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antagonist of the invention. A calibrated lens micrometer is used by a blinded observer to determine the distance of the wound gap.

Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is considered significant.

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

Example 29: Lymphadema Animal Model

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The purpose of this experimental approach is to create an appropriate and consistent lymphedema model for testing the therapeutic effects of an agonist or antagonist of the invention in lymphangiogenesis and re-establishment of the lymphatic circulatory system in the rat hind limb. Effectiveness is measured by swelling volume of the affected limb, quantification of the amount of lymphatic vasculature, total blood plasma protein, and histopathology. Acute lymphedema is observed for 7-10 days. Perhaps more importantly, the chronic progress of the edema is followed for up to 3-4 weeks.

Prior to beginning surgery, blood sample is drawn for protein concentration analysis. Male rats weighing approximately ~350g are dosed with Pentobarbital. Subsequently, the right legs are shaved from knee to hip. The shaved area is swabbed with gauze soaked in 70% EtOH. Blood is drawn for serum total protein testing. Circumference and volumetric measurements are made prior to injecting dye into paws after marking 2 measurement levels (0.5 cm above heel, at mid-pt of dorsal paw). The intradermal dorsum of both right and left paws are injected with 0.05 ml of 1% Evan's Blue. Circumference and volumetric measurements are then made following injection of dye into paws.

Using the knee joint as a landmark, a mid-leg inguinal incision is made circumferentially allowing the femoral vessels to be located. Forceps and hemostats are used to dissect and separate the skin flaps. After locating the femoral vessels, the lymphatic vessel that runs along side and underneath the vessel(s) is located. The main lymphatic vessels in this area are then electrically coagulated or suture ligated.

Using a microscope, muscles in back of the leg (near the semitendinosis and adductors) are bluntly dissected. The popliteal lymph node is then located. The 2 proximal

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and 2 distal lymphatic vessels and distal blood supply of the popliteal node are then and ligated by suturing. The popliteal lymph node, and any accompanying adipose tissue, is then removed by cutting connective tissues.

Care is taken to control any mild bleeding resulting from this procedure. After lymphatics are occluded, the skin flaps are sealed by using liquid skin (Vetbond) (AJ Buck). The separated skin edges are sealed to the underlying muscle tissue while leaving a gap of ~0.5 cm around the leg. Skin also may be anchored by suturing to underlying muscle when necessary.

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To avoid infection, animals are housed individually with mesh (no bedding). Recovering animals are checked daily through the optimal edematous peak, which typically occurred by day 5-7. The plateau edematous peak are then observed. To evaluate the intensity of the lymphedema, the circumference and volumes of 2 designated places on each paw before operation and daily for 7 days are measured. The effect plasma proteins on lymphedema is determined and whether protein analysis is a useful testing perimeter is also investigated. The weights of both control and edematous limbs are evaluated at 2 places. Analysis is performed in a blind manner.

Circumference Measurements: Under brief gas anesthetic to prevent limb movement, a cloth tape is used to measure limb circumference. Measurements are done at the ankle bone and dorsal paw by 2 different people then those 2 readings are averaged. Readings are taken from both control and edematous limbs.

Volumetric Measurements: On the day of surgery, animals are anesthetized with Pentobarbital and are tested prior to surgery. For daily volumetrics animals are under brief halothane anesthetic (rapid immobilization and quick recovery), both legs are shaved and equally marked using waterproof marker on legs. Legs are first dipped in water, then dipped into instrument to each marked level then measured by Buxco edema software(Chen/Victor). Data is recorded by one person, while the other is dipping the limb to marked area.

Blood-plasma protein measurements: Blood is drawn, spun, and serum separated prior to surgery and then at conclusion for total protein and Ca2+ comparison.

Limb Weight Comparison: After drawing blood, the animal is prepared for tissue collection. The limbs are amputated using a quillitine, then both experimental and control legs are cut at the ligature and weighed. A second weighing is done as the tibio-cacaneal joint is disarticulated and the foot is weighed.

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Histological Preparations: The transverse muscle located behind the knee (popliteal) area is dissected and arranged in a metal mold, filled with freezeGel, dipped into cold methylbutane, placed into labeled sample bags at - 80EC until sectioning. Upon sectioning, the muscle is observed under fluorescent microscopy for lymphatics..

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

Example 30: Suppression of TNF alpha-induced adhesion molecule expression by a Agonist or Antagonist of the Invention

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

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Tumor necrosis factor alpha (TNF-a), a potent proinflammatory cytokine, is a stimulator of all three CAMs on endothelial cells and may be involved in a wide variety of inflammatory responses, often resulting in a pathological outcome.

The potential of an agonist or antagonist of the invention to mediate a suppression of TNF-a induced CAM expression can be examined. A modified ELISA assay which uses ECs as a solid phase absorbent is employed to measure the amount of CAM expression on TNF-a treated ECs when co-stimulated with a member of the FGF family of proteins.

To perform the experiment, human umbilical vein endothelial cell (HUVEC) cultures are obtained from pooled cord harvests and maintained in growth medium (EGM-2; Clonetics, San Diego, CA) supplemented with 10% FCS and 1% penicillin/streptomycin in a 37 degree C humidified incubator containing 5% CO₂. HUVECs are seeded in 96-well

plates at concentrations of 1×10^4 cells/well in EGM medium at 37 degree C for 18-24 hrs or until confluent. The monolayers are subsequently washed 3 times with a serum-free solution of RPMI-1640 supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin, and treated with a given cytokine and/or growth factor(s) for 24 h at 37 degree C. Following incubation, the cells are then evaluated for CAM expression.

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Human Umbilical Vein Endothelial cells (HUVECs) are grown in a standard 96 well plate to confluence. Growth medium is removed from the cells and replaced with 90 ul of 199 Medium (10% FBS). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10 ul volumes). Plates are incubated at 37 degree C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100 µl of 0.1% paraformaldehyde-PBS(with Ca++ and Mg++) is added to each well. Plates are held at 4°C for 30 min.

Fixative is then removed from the wells and wells are washed 1X with PBS(+Ca,Mg)+0.5% BSA and drained. Do not allow the wells to dry. Add 10 μl of diluted primary antibody to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10 μg/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA.

Then add 20 μ l of diluted ExtrAvidin-Alkaline Phosphotase (1:5,000 dilution) to each well and incubated at 37°C for 30 min. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA. I tablet of p-Nitrophenol Phosphate pNPP is dissolved in 5 ml of glycine buffer (pH 10.4). 100 μ l of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphotase in glycine buffer: 1:5,000 (10°) > 10°-0.5 > 10°-1 > 10°-1.5 .5 μ l of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 μ l of pNNP reagent must then be added to each of the standard wells. The plate must be incubated at 37°C for 4h. A volume of 50 μ l of 3M NaOH is added to all wells. The results are quantified on a plate reader at 405 nm. The background subtraction option is used on blank wells filled with glycine buffer only. The template is set up to indicate the concentration of AP-conjugate in each standard well [5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

The studies described in this example tested activity of agonists or antagonists of the

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invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

Example 31: Production Of Polypeptide of the Invention For High-Throughput Screening

5 Assays

The following protocol produces a supernatant containing polypeptide of the present invention to be tested. This supernatant can then be used in the Screening Assays described in Examples 33-42.

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First, dilute Poly-D-Lysine (644 587 Boehringer-Mannheim) stock solution (1mg/ml in PBS) 1:20 in PBS (w/o calcium or magnesium 17-516F Biowhittaker) for a working solution of 50ug/ml. Add 200 ul of this solution to each well (24 well plates) and incubate at RT for 20 minutes. Be sure to distribute the solution over each well (note: a 12-channel pipetter may be used with tips on every other channel). Aspirate off the Poly-D-Lysine solution and rinse with 1ml PBS (Phosphate Buffered Saline). The PBS should remain in the well until just prior to plating the cells and plates may be poly-lysine coated in advance for up to two weeks.

Plate 293T cells (do not carry cells past P+20) at 2 x 10⁵ cells/well in .5ml DMEM(Dulbecco's Modified Eagle Medium)(with 4.5 G/L glucose and L-glutamine (12-604F Biowhittaker))/10% heat inactivated FBS(14-503F Biowhittaker)/1x Penstrep(17-602E Biowhittaker). Let the cells grow overnight.

The next day, mix together in a sterile solution basin: 300 ul Lipofectamine (18324-012 Gibco/BRL) and 5ml Optimem I (31985070 Gibco/BRL)/96-well plate. With a small volume multi-channel pipetter, aliquot approximately 2ug of an expression vector containing a polynucleotide insert, produced by the methods described in Examples 8-10, into an appropriately labeled 96-well round bottom plate. With a multi-channel pipetter, add 50ul of the Lipofectamine/Optimem I mixture to each well. Pipette up and down gently to mix. Incubate at RT 15-45 minutes. After about 20 minutes, use a multi-channel pipetter to add 150ul Optimem I to each well. As a control, one plate of vector DNA lacking an insert should be transfected with each set of transfections.

Preferably, the transfection should be performed by tag-teaming the following tasks. By tag-teaming, hands on time is cut in half, and the cells do not spend too much time on

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PBS. First, person A aspirates off the media from four 24-well plates of cells, and then person B rinses each well with .5-1ml PBS. Person A then aspirates off PBS rinse, and person B, using a12-channel pipetter with tips on every other channel, adds the 200ul of DNA/Lipofectamine/Optimem I complex to the odd wells first, then to the even wells, to each row on the 24-well plates. Incubate at 37 degree C for 6 hours.

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While cells are incubating, prepare appropriate media, either 1%BSA in DMEM with 1x penstrep, or HGS CHO-5 media (116.6 mg/L of CaCl2 (anhyd); 0.00130 mg/L CuSO₄-5H₂O; 0.050 mg/L of Fe(NO₃)₃-9H₂O; 0.417 mg/L of FeSO₄-7H₂O; 311.80 mg/L of Kcl; 28.64 mg/L of MgCl₂; 48.84 mg/L of MgSO₄; 6995.50 mg/L of NaCl; 2400.0 mg/L of 10 NaHCO3; 62.50 mg/L of NaH2PO4-H2O; 71.02 mg/L of Na2HPO4; .4320 mg/L of ZnSO4-7H₂O; .002 mg/L of Arachidonic Acid; 1.022 mg/L of Cholesterol; .070 mg/L of DL-alpha-Tocopherol-Acetate; 0.0520 mg/L of Linoleic Acid; 0.010 mg/L of Linolenic Acid; 0.010 mg/L of Myristic Acid; 0.010 mg/L of Oleic Acid; 0.010 mg/L of Palmitric Acid; 0.010 mg/L of Palmitic Acid; 100 mg/L of Pluronic F-68; 0.010 mg/L of Stearic Acid; 2.20 mg/L of 15 Tween 80; 4551 mg/L of D-Glucose; 130.85 mg/ml of L- Alanine; 147.50 mg/ml of L-Arginine-HCL; 7.50 mg/ml of L-Asparagine-H20; 6.65 mg/ml of L-Aspartic Acid; 29.56 mg/ml of L-Cystine-2HCL-H₂0; 31.29 mg/ml of L-Cystine-2HCL; 7.35 mg/ml of L-Glutamic Acid; 365.0 mg/ml of L-Glutamine; 18.75 mg/ml of Glycine; 52.48 mg/ml of L-Histidine-HCL-H₂0; 106.97 mg/ml of L-Isoleucine; 111.45 mg/ml of L-Leucine; 163.75 20 mg/ml of L-Lysine HCL; 32.34 mg/ml of L-Methionine; 68.48 mg/ml of L-Phenylalainine; 40.0 mg/ml of L-Proline; 26.25 mg/ml of L-Serine; 101.05 mg/ml of L-Threonine; 19.22 mg/ml of L-Tryptophan; 91.79 mg/ml of L-Tryrosine-2Na-2H20; and 99.65 mg/ml of L-Valine; 0.0035 mg/L of Biotin; 3.24 mg/L of D-Ca Pantothenate; 11.78 mg/L of Choline Chloride; 4.65 mg/L of Folic Acid; 15.60 mg/L of i-Inositol; 3.02 mg/L of Niacinamide; 3.00 25 mg/L of Pyridoxal HCL; 0.031 mg/L of Pyridoxine HCL; 0.319 mg/L of Riboflavin; 3.17 mg/L of Thiamine HCL; 0.365 mg/L of Thymidine; 0.680 mg/L of Vitamin B₁₂; 25 mM of HEPES Buffer; 2.39 mg/L of Na Hypoxanthine; 0.105 mg/L of Lipoic Acid; 0.081 mg/L of Sodium Putrescine-2HCL; 55.0 mg/L of Sodium Pyruvate; 0.0067 mg/L of Sodium Selenite; 20uM of Ethanolamine; 0.122 mg/L of Ferric Citrate; 41.70 mg/L of Methyl-B-Cyclodextrin complexed with Linoleic Acid; 33.33 mg/L of Methyl-B-Cyclodextrin complexed with Oleic Acid; 10 mg/L of Methyl-B-Cyclodextrin complexed with Retinal Acetate. Adjust

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osmolarity to 327 mOsm) with 2mm glutamine and 1x penstrep. (BSA (81-068-3 Bayer) 100gm dissolved in 1L DMEM for a 10% BSA stock solution). Filter the media and collect 50 ul for endotoxin assay in 15ml polystyrene conical.

The transfection reaction is terminated, preferably by tag-teaming, at the end of the incubation period. Person A aspirates off the transfection media, while person B adds 1.5ml appropriate media to each well. Incubate at 37 degree C for 45 or 72 hours depending on the media used: 1%BSA for 45 hours or CHO-5 for 72 hours.

On day four, using a 300ul multichannel pipetter, aliquot 600ul in one 1ml deep well plate and the remaining supernatant into a 2ml deep well. The supernatants from each well can then be used in the assays described in Examples 33-40.

It is specifically understood that when activity is obtained in any of the assays described below using a supernatant, the activity originates from either the polypeptide of the present invention directly (e.g., as a secreted protein) or by polypeptide of the present invention inducing expression of other proteins, which are then secreted into the supernatant. Thus, the invention further provides a method of identifying the protein in the supernatant characterized by an activity in a particular assay.

Example 32: Construction of GAS Reporter Construct

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One signal transduction pathway involved in the differentiation and proliferation of cells is called the Jaks-STATs pathway. Activated proteins in the Jaks-STATs pathway bind to gamma activation site "GAS" elements or interferon-sensitive responsive element ("ISRE"), located in the promoter of many genes. The binding of a protein to these elements alter the expression of the associated gene.

GAS and ISRE elements are recognized by a class of transcription factors called Signal Transducers and Activators of Transcription, or "STATs." There are six members of the STATs family. Stat1 and Stat3 are present in many cell types, as is Stat2 (as response to IFN-alpha is widespread). Stat4 is more restricted and is not in many cell types though it has been found in T helper class I, cells after treatment with IL-12. Stat5 was originally called mammary growth factor, but has been found at higher concentrations in other cells including myeloid cells. It can be activated in tissue culture cells by many cytokines.

The STATs are activated to translocate from the cytoplasm to the nucleus upon

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tyrosine phosphorylation by a set of kinases known as the Janus Kinase ("Jaks") family. Jaks represent a distinct family of soluble tyrosine kinases and include Tyk2, Jak1, Jak2, and Jak3. These kinases display significant sequence similarity and are generally catalytically inactive in resting cells.

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The Jaks are activated by a wide range of receptors summarized in the Table below. (Adapted from review by Schidler and Darnell, Ann. Rev. Biochem. 64:621-51 (1995).) A cytokine receptor family, capable of activating Jaks, is divided into two groups: (a) Class 1 includes receptors for IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11, IL-12, IL-15, Epo, PRL, GH, G-CSF, GM-CSF, LIF, CNTF, and thrombopoietin; and (b) Class 2 includes IFN-a, IFN-g, and IL-10. The Class 1 receptors share a conserved cysteine motif (a set of four conserved cysteines and one tryptophan) and a WSXWS motif (a membrane proximal region encoding Trp-Ser-Xxx-Trp-Ser (SEQ ID NO:1686)).

Thus, on binding of a ligand to a receptor, Jaks are activated, which in turn activate STATs, which then translocate and bind to GAS elements. This entire process is encompassed in the Jaks-STATs signal transduction pathway.

Therefore, activation of the Jaks-STATs pathway, reflected by the binding of the GAS or the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells. For example, growth factors and cytokines are known to activate the Jaks-STATs pathway. (See Table below.) Thus, by using GAS elements linked to reporter molecules, activators of the Jaks-STATs pathway can be identified.

			<u>JAKs</u>			STATS GAS	(elements) or ISRE
	<u>Ligand</u>	tyk2	Jak 1	Jak2	Jak3		
	IFAT C						
5	IFN family						
3	IFN-a/B	+	+	-	-	1,2,3	ISRE
	IFN-g		. +	+	•	I	GAS
	(IRF1>Lys6>IFP)						
	II-10	+	?	?	-	1,3	
10	gp130 family						
	IL-6 (Pleiotrohic)	+	+	+	?	1,3	GAS
	(IRF1>Lys6>IFP)						
	Il-11(Pleiotrohic)	?	+	?	?	1,3	
	OnM(Pleiotrohic)	?	+	+	?	1,3	
15	LIF(Pleiotrohic)	?	+	+	?	1,3	
	CNTF(Pleiotrohic)	-/+	+	+	?	1,3	
	G-CSF(Pleiotrohic)	?	+	?	?	1,3	
	IL-12(Pleiotrohic)	+	-	+	+	1,3	
20	g-C family						
	IL-2 (lymphocytes)		_			125	0.10
	IL-4 (lymph/myeloid)	-	+	-	+	1,3,5	GAS
	>>Ly6)(IgH)	-	+	-	+	6	GAS (IRF1 = IFP)
	IL-7 (lymphocytes)					, .	0.0
25	IL-9 (lymphocytes)	-	+	•	+	5	GAS
25		-	+	-	+	5	GAS
	IL-13 (lymphocyte) IL-15	•	+	?	?	6	GAS
	IL-15	?	+	?	+	5	GAS
	gp140 family						
30	IL-3 (myeloid)	-	-	+	-	5	GAS
	(IRF1>IFP>>Ly6)	•					
	IL-5 (myeloid)	-	-	+	-	5	GAS
	GM-CSF (myeloid)	-	-	+	-	5	GAS

	Growth hormone fam	ily					
	GH	?	-	+	-	5	
	PRL	?	+/-	+	-	1,3,5	
5	EPO	?	-	+	•	5	GAS(B-
	CAS>IRFI=IFP>>Ly	6)					
	Receptor Tyrosine Kir	nases					
	EGF	?	+	+	-	1,3	GAS (IRF1)
10							•
	PDGF	?	+	+	•	1,3	
	CSF-1	?	+	+	-	1,3	GAS (not IRF1)

To construct a synthetic GAS containing promoter element, which is used in the Biological Assays described in Examples 33-34, a PCR based strategy is employed to generate a GAS-SV40 promoter sequence. The 5' primer contains four tandem copies of the GAS binding site found in the IRF1 promoter and previously demonstrated to bind STATs upon induction with a range of cytokines (Rothman et al., Immunity 1:457-468 (1994).), although other GAS or ISRE elements can be used instead. The 5' primer also contains 18bp of sequence complementary to the SV40 early promoter sequence and is flanked with an Xhol site. The sequence of the 5' primer is:

10 5':GCGCCTCGAGATTTCCCCGAAATCTAGATTTCCCCGAAATGATTTCCCC GAAATGATTTCCCCGAAATATCTGCCATCTCAATTAG:3' (SEQ ID NO:1687)

The downstream primer is complementary to the SV40 promoter and is flanked with a Hind III site: 5':GCGGCAAGCTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO:1688)

PCR amplification is performed using the SV40 promoter template present in the B-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with Xhol/Hind III and subcloned into BLSK2-. (Stratagene.) Sequencing with forward and reverse primers confirms that the insert contains the following sequence:

With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or "SEAP." Clearly, however, any reporter molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter molecules that can be used instead of SEAP include chloramphenicol

acetyltransferase (CAT), luciferase, alkaline phosphatase, B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.

The above sequence confirmed synthetic GAS-SV40 promoter element is subcloned into the pSEAP-Promoter vector obtained from Clontech using HindlII and XhoI, effectively replacing the SV40 promoter with the amplified GAS:SV40 promoter element, to create the GAS-SEAP vector. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

Thus, in order to generate mammalian stable cell lines expressing the GAS-SEAP reporter, the GAS-SEAP cassette is removed from the GAS-SEAP vector using SalI and NotI, and inserted into a backbone vector containing the neomycin resistance gene, such as pGFP-1 (Clontech), using these restriction sites in the multiple cloning site, to create the GAS-SEAP/Neo vector. Once this vector is transfected into mammalian cells, this vector can then be used as a reporter molecule for GAS binding as described in Examples 33-34.

Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing NFK-B and EGR promoter sequences are described in Examples 35 and 36. However, many other promoters can be substituted using the protocols described in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/NF-KB/EGR, GAS/NF-KB, Il-2/NFAT, or NF-KB/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as HELA (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

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Example 33: High-Throughput Screening Assay for T-cell Activity.

The following protocol is used to assess T-cell activity by identifying factors, and determining whether supernate containing a polypeptide of the invention proliferates and/or differentiates T-cells. T-cell activity is assessed using the

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GAS/SEAP/Neo construct produced in Example 32. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The T-cell used in this assay is Jurkat T-cells (ATCC Accession No. TIB-152), although Molt-3 cells (ATCC Accession No. CRL-1552) and Molt-4 cells (ATCC Accession No. CRL-1582) cells can also be used.

Jurkat T-cells are lymphoblastic CD4+ Th1 helper cells. In order to generate stable cell lines, approximately 2 million Jurkat cells are transfected with the GAS-SEAP/neo vector using DMRIE-C (Life Technologies)(transfection procedure described below). The transfected cells are seeded to a density of approximately 20,000 cells per well and transfectants resistant to 1 mg/ml genticin selected. Resistant colonies are expanded and then tested for their response to increasing concentrations of interferon gamma. The dose response of a selected clone is demonstrated.

Specifically, the following protocol will yield sufficient cells for 75 wells containing 200 ul of cells. Thus, it is either scaled up, or performed in multiple to generate sufficient cells for multiple 96 well plates. Jurkat cells are maintained in RPMI + 10% serum with 1%Pen-Strep. Combine 2.5 mls of OPTI-MEM (Life Technologies) with 10 ug of plasmid DNA in a T25 flask. Add 2.5 ml OPTI-MEM containing 50 ul of DMRIE-C and incubate at room temperature for 15-45 mins.

During the incubation period, count cell concentration, spin down the required number of cells (10^7 per transfection), and resuspend in OPTI-MEM to a final concentration of 10^7 cells/ml. Then add 1ml of 1 x 10^7 cells in OPTI-MEM to T25 flask and incubate at 37 degree C for 6 hrs. After the incubation, add 10 ml of RPMI + 15% serum.

The Jurkat:GAS-SEAP stable reporter lines are maintained in RPMI + 10% serum, 1 mg/ml Genticin, and 1% Pen-Strep. These cells are treated with supernatants containing polypeptide of the present invention or polypeptide of the present invention induced polypeptides as produced by the protocol described in Example 31.

On the day of treatment with the supernatant, the cells should be washed and

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resuspended in fresh RPMI + 10% serum to a density of 500,000 cells per ml. The exact number of cells required will depend on the number of supernatants being screened. For one 96 well plate, approximately 10 million cells (for 10 plates, 100 million cells) are required.

Transfer the cells to a triangular reservoir boat, in order to dispense the cells into a 96 well dish, using a 12 channel pipette. Using a 12 channel pipette, transfer 200 ul of cells into each well (therefore adding 100, 000 cells per well).

After all the plates have been seeded, 50 ul of the supernatants are transferred directly from the 96 well plate containing the supernatants into each well using a 12 channel pipette. In addition, a dose of exogenous interferon gamma (0.1, 1.0, 10 ng) is added to wells H9, H10, and H11 to serve as additional positive controls for the assay.

The 96 well dishes containing Jurkat cells treated with supernatants are placed in an incubator for 48 hrs (note: this time is variable between 48-72 hrs). 35 ul samples from each well are then transferred to an opaque 96 well plate using a 12 channel pipette. The opaque plates should be covered (using sellophene covers) and stored at -20 degree C until SEAP assays are performed according to Example 37. The plates containing the remaining treated cells are placed at 4 degree C and serve as a source of material for repeating the assay on a specific well if desired.

As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate Jurkat T cells. Over 30 fold induction is typically observed in the positive control wells.

The above protocol may be used in the generation of both transient, as well as, stable transfected cells, which would be apparent to those of skill in the art.

Example 34: High-Throughput Screening Assay Identifying Myeloid Activity

The following protocol is used to assess myeloid activity of polypeptide of the present invention by determining whether polypeptide of the present invention proliferates and/or differentiates myeloid cells. Myeloid cell activity is assessed using

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the GAS/SEAP/Neo construct produced in Example 32. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The myeloid cell used in this assay is U937, a pre-monocyte cell line, although TF-1, HL60, or KG1 can be used.

To transiently transfect U937 cells with the GAS/SEAP/Neo construct produced in Example 32, a DEAE-Dextran method (Kharbanda et. al., 1994, Cell Growth & Differentiation, 5:259-265) is used. First, harvest 2x10e⁷ U937 cells and wash with PBS. The U937 cells are usually grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin.

Next, suspend the cells in 1 ml of 20 mM Tris-HCl (pH 7.4) buffer containing 0.5 mg/ml DEAE-Dextran, 8 ug GAS-SEAP2 plasmid DNA, 140 mM NaCl, 5 mM KCl, 375 uM Na₂HPO₄.7H₂O, 1 mM MgCl₂, and 675 uM CaCl₂. Incubate at 37 degrees C for 45 min.

Wash the cells with RPMI 1640 medium containing 10% FBS and then resuspend in 10 ml complete medium and incubate at 37 degree C for 36 hr.

The GAS-SEAP/U937 stable cells are obtained by growing the cells in 400 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 400 ug/ml G418 for couple of passages.

These cells are tested by harvesting $1x10^8$ cells (this is enough for ten 96-well plates assay) and wash with PBS. Suspend the cells in 200 ml above described growth medium, with a final density of $5x10^5$ cells/ml. Plate 200 ul cells per well in the 96-well plate (or $1x10^5$ cells/well).

Add 50 ul of the supernatant prepared by the protocol described in Example 31. Incubate at 37 degee C for 48 to 72 hr. As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate U937 cells. Over 30 fold induction is typically observed in the positive control wells. SEAP assay the supernatant according to the protocol described in Example 37.

30 Example 35: High-Throughput Screening Assay Identifying Neuronal Activity.

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When cells undergo differentiation and proliferation, a group of genes are activated through many different signal transduction pathways. One of these genes, EGR1 (early growth response gene 1), is induced in various tissues and cell types upon activation. The promoter of EGR1 is responsible for such induction. Using the EGR1 promoter linked to reporter molecules, activation of cells can be assessed by polypeptide of the present invention.

Particularly, the following protocol is used to assess neuronal activity in PC12 cell lines. PC12 cells (rat phenochromocytoma cells) are known to proliferate and/or differentiate by activation with a number of mitogens, such as TPA (tetradecanoyl phorbol acetate), NGF (nerve growth factor), and EGF (epidermal growth factor). The EGR1 gene expression is activated during this treatment. Thus, by stably transfecting PC12 cells with a construct containing an EGR promoter linked to SEAP reporter, activation of PC12 cells by polypeptide of the present invention can be assessed.

The EGR/SEAP reporter construct can be assembled by the following protocol. The EGR-1 promoter sequence (-633 to +1)(Sakamoto K et al., Oncogene 6:867-871 (1991)) can be PCR amplified from human genomic DNA using the following primers:

- 5' GCGCTCGAGGGATGACAGCGATAGAACCCCGG -3' (SEQ ID NO: 1690)
 - 5' GCGAAGCTTCGCGACTCCCGGATCCGCCTC-3' (SEQ ID NO: 1691)

Using the GAS:SEAP/Neo vector produced in Example 32, EGR1 amplified product can then be inserted into this vector. Linearize the GAS:SEAP/Neo vector using restriction enzymes XhoI/HindIII, removing the GAS/SV40 stuffer. Restrict the EGR1 amplified product with these same enzymes. Ligate the vector and the EGR1 promoter.

To prepare 96 well-plates for cell culture, two mls of a coating solution (1:30 dilution of collagen type I (Upstate Biotech Inc. Cat#08-115) in 30% ethanol (filter

sterilized)) is added per one 10 cm plate or 50 ml per well of the 96-well plate, and allowed to air dry for 2 hr.

PC12 cells are routinely grown in RPMI-1640 medium (Bio Whittaker) containing 10% horse serum (JRH BIOSCIENCES, Cat. # 12449-78P), 5% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 ug/ml streptomycin on a precoated 10 cm tissue culture dish. One to four split is done every three to four days. Cells are removed from the plates by scraping and resuspended with pipetting up and down for more than 15 times.

Transfect the EGR/SEAP/Neo construct into PC12 using the Lipofectamine protocol described in Example 31. EGR-SEAP/PC12 stable cells are obtained by growing the cells in 300 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 300 ug/ml G418 for couple of passages.

To assay for neuronal activity, a 10 cm plate with cells around 70 to 80% confluent is screened by removing the old medium. Wash the cells once with PBS (Phosphate buffered saline). Then starve the cells in low serum medium (RPMI-1640 containing 1% horse serum and 0.5% FBS with antibiotics) overnight.

The next morning, remove the medium and wash the cells with PBS. Scrape off the cells from the plate, suspend the cells well in 2 ml low serum medium. Count the cell number and add more low serum medium to reach final cell density as 5×10^5 cells/ml.

Add 200 ul of the cell suspension to each well of 96-well plate (equivalent to 1×10^5 cells/well). Add 50 ul supernatant produced by Example 31, 37 degree C for 48 to 72 hr. As a positive control, a growth factor known to activate PC12 cells through EGR can be used, such as 50 ng/ul of Neuronal Growth Factor (NGF). Over fifty-fold induction of SEAP is typically seen in the positive control wells. SEAP assay the supernatant according to Example 37.

Example 36: High-Throughput Screening Assay for T-cell Activity

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NF-KB (Nuclear Factor KB) is a transcription factor activated by a wide variety of agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotoxin-alpha and lymphotoxin-beta, by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, NF-KB regulates the expression of genes involved in immune cell activation, control of apoptosis (NF- KB appears to shield cells from apoptosis), B and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

In non-stimulated conditions, NF- KB is retained in the cytoplasm with I-KB (Inhibitor KB). However, upon stimulation, I- KB is phosphorylated and degraded, causing NF- KB to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by NF- KB include IL-2, IL-6, GM-CSF, ICAM-1 and class 1 MHC.

Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the NF-KB promoter element are used to screen the supernatants produced in Example 31. Activators or inhibitors of NF-KB would be useful in treating, preventing, and/or diagnosing diseases. For example, inhibitors of NF-KB could be used to treat those diseases related to the acute or chronic activation of NF-KB, such as rheumatoid arthritis.

To construct a vector containing the NF-KB promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of the NF-KB binding site (GGGGACTTTCCC) (SEQ ID NO:1692), 18 bp of sequence complementary to the 5' end of the SV40 early promoter sequence, and is flanked with an XhoI site:

5':GCGGCCTCGAGGGGACTTTCCCGGGGACTTTCCGGGAC
TTTCCATCCTGCCATCTCAATTAG:3' (SEQ ID NO:1693)

The downstream primer is complementary to the 3' end of the SV40 promoter and is flanked with a Hind III site:

5':GCGGCAAGCTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO:1688)

PCR amplification is performed using the SV40 promoter template present in the pB-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is

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digested with XhoI and Hind III and subcloned into BLSK2-. (Stratagene) Sequencing with the T7 and T3 primers confirms the insert contains the following sequence:

5':CTCGAGGGGACTTTCCCGGGGACTTTCCCGGGACTTTCC
ATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCC
ATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGA
CTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTA
TTCCAGAAGTAGTGAGGAGGCCTTTTTTGGAGGCCTAGGCTTTTTGCAAAAA
GCTT:3' (SEQ ID NO:1694)

Next, replace the SV40 minimal promoter element present in the pSEAP2-promoter plasmid (Clontech) with this NF-KB/SV40 fragment using XhoI and HindIII. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

In order to generate stable mammalian cell lines, the NF-KB/SV40/SEAP cassette is removed from the above NF-KB/SEAP vector using restriction enzymes SalI and NotI, and inserted into a vector containing neomycin resistance. Particularly, the NF-KB/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP-1 with SalI and NotI.

Once NF-KB/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 33. Similarly, the method for assaying supernatants with these stable Jurkat T-cells is also described in Example 33. As a positive control, exogenous TNF alpha (0.1,1, 10 ng) is added to wells H9, H10, and H11, with a 5-10 fold activation typically observed.

25 Example 37: Assay for SEAP Activity

As a reporter molecule for the assays described in Examples 33-36, SEAP activity is assayed using the Tropix Phospho-light Kit (Cat. BP-400) according to the following general procedure. The Tropix Phospho-light Kit supplies the Dilution, Assay, and Reaction Buffers used below.

Prime a dispenser with the 2.5x Dilution Buffer and dispense 15 ul of 2.5x dilution buffer into Optiplates containing 35 ul of a supernatant. Seal the plates with a plastic sealer and incubate at 65 degree C for 30 min. Separate the Optiplates to avoid uneven heating.

Cool the samples to room temperature for 15 minutes. Empty the dispenser and prime with the Assay Buffer. Add 50 ml Assay Buffer and incubate at room temperature 5 min. Empty the dispenser and prime with the Reaction Buffer (see the table below).. Add 50 ul Reaction Buffer and incubate at room temperature for 20 minutes. Since the intensity of the chemiluminescent signal is time dependent, and it takes about 10 minutes to read 5 plates on luminometer, one should treat 5 plates at each time and start the second set 10 minutes later.

Read the relative light unit in the luminometer. Set H12 as blank, and print the results. An increase in chemiluminescence indicates reporter activity.

15 Reaction Buffer Formulation:

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# of plates	Rxn buffer diluent (ml)	CSPD (ml)
10	60	3
11	65	3.25
12	70	3.5
13	75	3.75
14	80	4
15	85	4.25
16	90	4.5
17	95	4.75
18	100	5
19	105	5.25
20	110	5.5
21	115	5.75
22	120	6

23	125	6.25
24	130	6.5
25	135	6.75
26	140	7
27	145	7.25
28	150	7.5
29	155	7.75
30	160	8
31	165	8.25
32	. 170	8.5
33	175	8.75
34	180	9
35	185	9.25
36	190	9.5
37	195	9.75
38	200	10
39	205	10.25
40	210	10.5
41	215	10.75
42	220	11
43	225	11.25
44	230	11.5
45	235	11.75
46	240	12
47	245	12.25
48	250	12.5
49	255	12.75
50	260	13

Example 38: High-Throughput Screening Assay Identifying Changes in Small

Molecule Concentration and Membrane Permeability

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Binding of a ligand to a receptor is known to alter intracellular levels of small molecules. such as calcium, potassium, sodium, and pH, as well as alter membrane potential. These alterations can be measured in an assay to identify supernatants which bind to receptors of a particular cell. Although the following protocol describes an assay for calcium, this protocol can easily be modified to detect changes in potassium, sodium, pH, membrane potential, or any other small molecule which is detectable by a fluorescent probe.

The following assay uses Fluorometric Imaging Plate Reader ("FLIPR") to measure changes in fluorescent molecules (Molecular Probes) that bind small molecules. Clearly, any fluorescent molecule detecting a small molecule can be used instead of the calcium fluorescent molecule, fluo-4 (Molecular Probes, Inc.; catalog no. F-14202), used here.

For adherent cells, seed the cells at 10,000 -20,000 cells/well in a Co-star black 96-well plate with clear bottom. The plate is incubated in a CO₂ incubator for 20 hours. The adherent cells are washed two times in Biotek washer with 200 ul of HBSS (Hank's Balanced Salt Solution) leaving 100 ul of buffer after the final wash.

A stock solution of 1 mg/ml fluo-4 is made in 10% pluronic acid DMSO. To load the cells with fluo-4, 50 ul of 12 ug/ml fluo-4 is added to each well. The plate is incubated at 37 degrees C in a CO₂ incubator for 60 min. The plate is washed four times in the Biotek washer with HBSS leaving 100 ul of buffer.

For non-adherent cells, the cells are spun down from culture media. Cells are re-suspended to 2-5x10⁶ cells/ml with HBSS in a 50-ml conical tube. 4 ul of 1 mg/ml fluo-4 solution in 10% pluronic acid DMSO is added to each ml of cell suspension. The tube is then placed in a 37 degrees C water bath for 30-60 min. The cells are washed twice with HBSS, resuspended to 1x10⁶ cells/ml, and dispensed into a microplate. 100 ul/well. The plate is centrifuged at 1000 rpm for 5 min. The plate is then washed once in Denley Cell Wash with 200 ul, followed by an aspiration step to 100 ul final volume.

For a non-cell based assay, each well contains a fluorescent molecule, such as fluo-4. The supernatant is added to the well, and a change in fluorescence is detected.

To measure the fluorescence of intracellular calcium, the FLIPR is set for the following parameters: (1) System gain is 300-800 mW; (2) Exposure time is 0.4 second; (3) Camera F/stop is F/2; (4) Excitation is 488 nm; (5) Emission is 530 nm; and (6) Sample addition is 50 ul. Increased emission at 530 nm indicates an extracellular signaling event caused by the a molecule, either polypeptide of the present invention or a molecule induced by polypeptide of the present invention, which has resulted in an increase in the intracellular Ca⁺⁺ concentration.

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Example 40: High-Throughput Screening Assay Identifying Tyrosine Kinase Activity

The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor subfamilies. In addition there are a large family of RPTKs for which the corresponding ligand is unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

Activation of RPTK by ligands involves ligand-mediated receptor dimerization, resulting in transphosphorylation of the receptor subunits and activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include receptor associated tyrosine kinases of the src-family (e.g., src, yes, lck, lyn, fyn) and non-receptor linked and cytosolic protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine superfamily of receptors (e.g., the Interleukins, Interferons, GM-CSF, and Leptin).

Because of the wide range of known factors capable of stimulating tyrosine kinase activity, identifying whether polypeptide of the present invention or a molecule induced by polypeptide of the present invention is capable of activating tyrosine

kinase signal transduction pathways is of interest. Therefore, the following protocol is designed to identify such molecules capable of activating the tyrosine kinase signal transduction pathways.

Seed target cells (e.g., primary keratinocytes) at a density of approximately 25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with 100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr with 100 ml of cell culture grade type I collagen (50 mg/ml), gelatin (2%) or polylysine (50 mg/ml), all of which can be purchased from Sigma Chemicals (St. Louis, MO) or 10% Matrigel purchased from Becton Dickinson (Bedford,MA), or calf serum, rinsed with PBS and stored at 4 degree C. Cell growth on these plates is assayed by seeding 5,000 cells/well in growth medium and indirect quantitation of cell number through use of alamarBlue as described by the manufacturer Alamar Biosciences, Inc. (Sacramento, CA) after 48 hr. Falcon plate covers #3071 from Becton Dickinson (Bedford,MA) are used to cover the Loprodyne Silent Screen Plates. Falcon Microtest III cell culture plates can also be used in some proliferation experiments.

To prepare extracts, A431 cells are seeded onto the nylon membranes of Loprodyne plates (20,000/200ml/well) and cultured overnight in complete medium. Cells are quiesced by incubation in serum-free basal medium for 24 hr. After 5-20 minutes treatment with EGF (60ng/ml) or 50 ul of the supernatant produced in Example 31, the medium was removed and 100 ml of extraction buffer ((20 mM HEPES pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na3VO4, 2 mM Na4P2O7 and a cocktail of protease inhibitors (# 1836170) obtained from Boeheringer Mannheim (Indianapolis, IN) is added to each well and the plate is shaken on a rotating shaker for 5 minutes at 4°C. The plate is then placed in a vacuum transfer manifold and the extract filtered through the 0.45 mm membrane bottoms of each well using house vacuum. Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum manifold and immediately placed on ice. To obtain extracts clarified by centrifugation, the content of each well, after

detergent solubilization for 5 minutes, is removed and centrifuged for 15 minutes at 4 degree C at 16,000 x g.

Test the filtered extracts for levels of tyrosine kinase activity. Although many methods of detecting tyrosine kinase activity are known, one method is described here.

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Generally, the tyrosine kinase activity of a supernatant is evaluated by determining its ability to phosphorylate a tyrosine residue on a specific substrate (a biotinylated peptide). Biotinylated peptides that can be used for this purpose include PSK1 (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34) and PSK2 (corresponding to amino acids 1-17 of gastrin). Both peptides are substrates for a range of tyrosine kinases and are available from Boehringer Mannheim.

The tyrosine kinase reaction is set up by adding the following components in order. First, add 10ul of 5uM Biotinylated Peptide, then 10ul ATP/Mg₂₊ (5mM ATP/50mM MgCl₂), then 10ul of 5x Assay Buffer (40mM imidazole hydrochloride, pH7.3, 40 mM beta-glycerophosphate, 1mM EGTA, 100mM MgCl₂, 5 mM MnCl₂, 0.5 mg/ml BSA), then 5ul of Sodium Vanadate(1mM), and then 5ul of water. Mix the components gently and preincubate the reaction mix at 30 degree C for 2 min. Initial the reaction by adding 10ul of the control enzyme or the filtered supernatant.

The tyrosine kinase assay reaction is then terminated by adding 10 ul of 120mm EDTA and place the reactions on ice.

Tyrosine kinase activity is determined by transferring 50 ul aliquot of reaction mixture to a microtiter plate (MTP) module and incubating at 37 degree C for 20 min. This allows the streptavadin coated 96 well plate to associate with the biotinylated peptide. Wash the MTP module with 300ul/well of PBS four times. Next add 75 ul of anti-phospotyrosine antibody conjugated to horse radish peroxidase(anti-P-Tyr-POD(0.5u/ml)) to each well and incubate at 37 degree C for one hour. Wash the well as above.

Next add 100ul of peroxidase substrate solution (Boehringer Mannheim) and incubate at room temperature for at least 5 mins (up to 30 min). Measure the absorbance of the sample at 405 nm by using ELISA reader. The level of bound

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peroxidase activity is quantitated using an ELISA reader and reflects the level of tyrosine kinase activity.

Example 41: High-Throughput Screening Assay Identifying Phosphorylation Activity

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As a potential alternative and/or compliment to the assay of protein tyrosine kinase activity described in Example 40, an assay which detects activation (phosphorylation) of major intracellular signal transduction intermediates can also be used. For example, as described below one particular assay can detect tyrosine phosphorylation of the Erk-1 and Erk-2 kinases. However, phosphorylation of other molecules, such as Raf, JNK, p38 MAP, Map kinase kinase (MEK), MEK kinase, Src, Muscle specific kinase (MuSK), IRAK, Tec, and Janus, as well as any other phosphoserine, phosphotyrosine, or phosphothreonine molecule, can be detected by substituting these molecules for Erk-1 or Erk-2 in the following assay.

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Specifically, assay plates are made by coating the wells of a 96-well ELISA plate with 0.1ml of protein G (1ug/ml) for 2 hr at room temp, (RT). The plates are then rinsed with PBS and blocked with 3% BSA/PBS for 1 hr at RT. The protein G plates are then treated with 2 commercial monoclonal antibodies (100ng/well) against Erk-1 and Erk-2 (1 hr at RT) (Santa Cruz Biotechnology). (To detect other molecules, this step can easily be modified by substituting a monoclonal antibody detecting any of the above described molecules.) After 3-5 rinses with PBS, the plates are stored at 4 degree C until use.

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A431 cells are seeded at 20,000/well in a 96-well Loprodyne filterplate and cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6ng/well) or 50 ul of the supernatants obtained in Example 31 for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.

After incubation with the extract for 1 hr at RT, the wells are again rinsed. As a positive control, a commercial preparation of MAP kinase (10ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit)

antibody (lug/ml) which specifically recognizes the phosphorylated epitope of the Erk-1 and Erk-2 kinases (1 hr at RT). This antibody is biotinylated by standard procedures. The bound polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased fluorescent signal over background indicates a phosphorylation by polypeptide of the present invention or a molecule induced by polypeptide of the present invention.

Example 42: Assay for the Stimulation of Bone Marrow CD34+ Cell Proliferation

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This assay is based on the ability of human CD34+ to proliferate in the presence of hematopoietic growth factors and evaluates the ability of isolated polypeptides expressed in mammalian cells to stimulate proliferation of CD34+ cells.

It has been previously shown that most mature precursors will respond to only a single signal. More immature precursors require at least two signals to respond. Therefore, to test the effect of polypeptides on hematopoietic activity of a wide range of progenitor cells, the assay contains a given polypeptide in the presence or absence of other hematopoietic growth factors. Isolated cells are cultured for 5 days in the presence of Stem Cell Factor (SCF) in combination with tested sample. SCF alone has a very limited effect on the proliferation of bone marrow (BM) cells, acting in such conditions only as a "survival" factor. However, combined with any factor exhibiting stimulatory effect on these cells (e.g., IL-3), SCF will cause a synergistic effect. Therefore, if the tested polypeptide has a stimulatory effect on a hematopoietic progenitors, such activity can be easily detected. Since normal BM cells have a low level of cycling cells, it is likely that any inhibitory effect of a given polypeptide, or agonists or antagonists thereof, might not be detected. Accordingly, assays for an inhibitory effect on progenitors is preferably tested in cells that are first subjected to in vitro stimulation with SCF+IL+3, and then contacted with the compound that is being evaluated for inhibition of such induced proliferation.

Briefly, CD34+ cells are isolated using methods known in the art. The cells

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are thawed and resuspended in medium (QBSF 60 serum-free medium with 1% L-glutamine (500ml) Quality Biological, Inc., Gaithersburg, MD Cat# 160-204-101). After several gentle centrifugation steps at 200 x g, cells are allowed to rest for one hour. The cell count is adjusted to 2.5×10^5 cells/ml. During this time, $100 \mu l$ of sterile water is added to the peripheral wells of a 96-well plate. The cytokines that can be tested with a given polypeptide in this assay is rhSCF (R&D Systems, Minneapolis, MN, Cat# 255-SC) at 50 ng/ml alone and in combination with rhSCF and rhIL-3 (R&D Systems, Minneapolis, MN, Cat# 203-ML) at 30 ng/ml. After one hour, $10 \mu l$ of prepared cytokines, $50 \mu l$ of the supernatants prepared in Example 31 (supernatants at 1:2 dilution = $50 \mu l$) and $20 \mu l$ of diluted cells are added to the media which is already present in the wells to allow for a final total volume of $100 \mu l$. The plates are then placed in a 37° C/5% CO₂ incubator for five days.

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Eighteen hours before the assay is harvested, $0.5~\mu\text{Ci/well}$ of [3H] Thymidine is added in a 10 μ l volume to each well to determine the proliferation rate. The experiment is terminated by harvesting the cells from each 96-well plate to a filtermat using the Tomtec Harvester 96. After harvesting, the filtermats are dried, trimmed and placed into OmniFilter assemblies consisting of one OmniFilter plate and one OmniFilter Tray. 60 μ l Microscint is added to each well and the plate sealed with TopSeal-A press-on sealing film A bar code 15 sticker is affixed to the first plate for counting. The sealed plates is then loaded and the level of radioactivity determined via the Packard Top Count and the printed data collected for analysis. The level of radioactivity reflects the amount of cell proliferation.

The studies described in this example test the activity of a given polypeptide to stimulate bone marrow CD34+ cell proliferation. One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof. As a nonlimiting example, potential antagonists tested in this assay would be expected to inhibit cell proliferation in the presence of cytokines and/or to increase the inhibition of cell proliferation in the presence of cytokines and a given polypeptide. In contrast, potential agonists tested in this assay would be expected to enhance cell

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proliferation and/or to decrease the inhibition of cell proliferation in the presence of cytokines and a given polypeptide.

The ability of a gene to stimulate the proliferation of bone marrow CD34+ cells indicates that polynucleotides and polypeptides corresponding to the gene are useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein.

Example 43: Assay for Extracellular Matrix Enhanced Cell Response (EMECR)

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The objective of the Extracellular Matrix Enhanced Cell Response (EMECR) assay is to identify gene products (e.g., isolated polypeptides) that act on the hematopoietic stem cells in the context of the extracellular matrix (ECM) induced signal.

Cells respond to the regulatory factors in the context of signal(s) received from the surrounding microenvironment. For example, fibroblasts, and endothelial and epithelial stem cells fail to replicate in the absence of signals from the ECM. Hematopoietic stem cells can undergo self-renewal in the bone marrow, but not in in vitro suspension culture. The ability of stem cells to undergo self-renewal in vitro is dependent upon their interaction with the stromal cells and the ECM protein fibronectin (fn). Adhesion of cells to fn is mediated by the $\alpha_5.\beta_1$ and $\alpha_4.\beta_1$ integrin receptors, which are expressed by human and mouse hematopoietic stem cells. The factor(s) which integrate with the ECM environment and responsible for stimulating stem cell self-renewal has not yet been identified. Discovery of such factors should be of great interest in gene therapy and bone marrow transplant applications

Briefly, polystyrene, non tissue culture treated, 96-well plates are coated with fn fragment at a coating concentration of $0.2~\mu g/~cm^2$. Mouse bone marrow cells are plated (1,000 cells/well) in 0.2 ml of serum-free medium. Cells cultured in the presence of IL-3 (5 ng/ml) + SCF (50 ng/ml) would serve as the positive control,

conditions under which little self-renewal but pronounced differentiation of the stem cells is to be expected. Gene products of the invention (e.g., including, but not limited to, polynucleotides and polypeptides of the present invention, and supernatants produced in Example 31), are tested with appropriate negative controls in the presence and absence of SCF(5.0 ng/ml), where test factor supernates represent 10% of the total assay volume. The plated cells are then allowed to grow by incubating in a low oxygen environment (5% CO₂, 7% O₂, and 88% N₂) tissue culture incubator for 7 days. The number of proliferating cells within the wells is then quantitated by measuring thymidine incorporation into cellular DNA. Verification of the positive hits in the assay will require phenotypic characterization of the cells, which can be accomplished by scaling up of the culture system and using appropriate antibody reagents against cell surface antigens and FACScan.

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One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

If a particular polypeptide of the present invention is found to be a stimulator of hematopoietic progenitors, polynucleotides and polypeptides corresponding to the gene encoding said polypeptide may be useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein. The gene product may also be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Additionally, the polynucleotides and/or polypeptides of the gene of interest and/or agonists and/or antagonists thereof, may also be employed to inhibit the proliferation and differentiation of hematopoietic cells and therefore may be employed to protect bone marrow stem cells from chemotherapeutic agents during chemotherapy. This antiproliferative effect may allow administration of higher doses of chemotherapeutic agents and, therefore, more effective chemotherapeutic treatment.

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Moreover, polynucleotides and polypeptides corresponding to the gene of interest may also be useful for the treatment and diagnosis of hematopoietic related disorders such as, for example, anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex-vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia.

Example 44: Human Dermal Fibroblast and Aortic Smooth Muscle Cell Proliferation

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The polypeptide of interest is added to cultures of normal human dermal fibroblasts (NHDF) and human aortic smooth muscle cells (AoSMC) and two coassays are performed with each sample. The first assay examines the effect of the polypeptide of interest on the proliferation of normal human dermal fibroblasts (NHDF) or aortic smooth muscle cells (AoSMC). Aberrant growth of fibroblasts or smooth muscle cells is a part of several pathological processes, including fibrosis, and restenosis. The second assay examines IL6 production by both NHDF and SMC. IL6 production is an indication of functional activation. Activated cells will have increased production of a number of cytokines and other factors, which can result in a proinflammatory or immunomodulatory outcome. Assays are run with and without co-TNFa stimulation, in order to check for costimulatory or inhibitory activity.

Briefly, on day 1, 96-well black plates are set up with 1000 cells/well (NHDF) or 2000 cells/well (AoSMC) in 100 μl culture media. NHDF culture media contains: Clonetics FB basal media, 1mg/ml hFGF, 5mg/ml insulin, 50mg/ml gentamycin, 2%FBS, while AoSMC culture media contains Clonetics SM basal media, 0.5 μg/ml hEGF, 5mg/ml insulin, 1μg/ml hFGF, 50mg/ml gentamycin, 50 μg/ml Amphotericin B, 5%FBS. After incubation at 37°C for at least 4-5 hours, culture media is aspirated and replaced with growth arrest media. Growth arrest media for NHDF contains fibroblast basal media, 50mg/ml gentamycin, 2% FBS, while growth arrest media for AoSMC contains SM basal media, 50mg/ml gentamycin, 50μg/ml Amphotericin B,

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0.4% FBS. Incubate at 37°C until day 2.

On day 2, serial dilutions and templates of the polypeptide of interest are designed such that they always include media controls and known-protein controls. For both stimulation and inhibition experiments, proteins are diluted in growth arrest media. For inhibition experiments, TNFa is added to a final concentration of 2ng/ml (NHDF) or 5ng/ml (AoSMC). Add 1/3 vol media containing controls or polypeptides of the present invention and incubate at 37°C/5% CO₂ until day 5.

Transfer $60\mu l$ from each well to another labeled 96-well plate, cover with a plate-sealer, and store at 4°C until Day 6 (for IL6 ELISA). To the remaining $100 \mu l$ in the cell culture plate, aseptically add Alamar Blue in an amount equal to 10% of the culture volume ($10\mu l$). Return plates to incubator for 3 to 4 hours. Then measure fluorescence with excitation at 530nm and emission at 590nm using the CytoFluor. This yields the growth stimulation/inhibition data.

On day 5, the IL6 ELISA is performed by coating a 96 well plate with 50-100 ul/well of Anti-Human IL6 Monoclonal antibody diluted in PBS, pH 7.4, incubate ON at room temperature.

On day 6, empty the plates into the sink and blot on paper towels. Prepare Assay Buffer containing PBS with 4% BSA. Block the plates with 200 µl/well of Pierce Super Block blocking buffer in PBS for 1-2 hr and then wash plates with wash buffer (PBS, 0.05% Tween-20). Blot plates on paper towels. Then add 50 µl/well of diluted Anti-Human IL-6 Monoclonal, Biotin-labeled antibody at 0.50 mg/ml. Make dilutions of IL-6 stock in media (30, 10, 3, 1, 0.3, 0 ng/ml). Add duplicate samples to top row of plate. Cover the plates and incubate for 2 hours at RT on shaker. Plates are washed with wash buffer and blotted on paper towels. Dilute EU-labeled Streptavidin 1:1000 in Assay buffer, and add 100 µl/well. Cover the plate and incubate 1 h at RT. Plates are again washed with wash buffer and blotted on paper towels. Add 100 µl/well of Enhancement Solution and shake for 5 minutes. Read the plate on the Wallac DELFIA Fluorometer. Readings from triplicate samples in each assay are tabulated and averaged.

A positive result in this assay suggests AoSMC cell proliferation and that the

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polypeptide of the present invention may be involved in dermal fibroblast proliferation and/or smooth muscle cell proliferation. A positive result also suggests many potential uses of polypeptides, polynucleotides, agonists and/or antagonists of the polynucleotide/polypeptide of the present invention which gives a positive result. For example, inflammation and immune responses, wound healing, and angiogenesis, as detailed throughout this specification. Particularly, polypeptides of the present invention and polynucleotides of the present invention may be used in wound healing and dermal regeneration, as well as the promotion of vasculargenesis, both of the blood vessels and lymphatics. The growth of vessels can be used in the treatment of, for example, cardiovascular diseases. Additionally, antagonists of polypeptides and polynucleotides of the invention may be useful in treating diseases, disorders, and/or conditions which involve angiogenesis by acting as an anti-vascular (e.g., antiangiogenesis). These diseases, disorders, and/or conditions are known in the art and/or are described herein, such as, for example, malignancies, solid tumors, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; artheroscleric plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uvietis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis. Moreover, antagonists of polypeptides and polynucleotides of the invention may be useful in treating anti-hyperproliferative diseases and/or anti-inflammatory known in the art and/or described herein.

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One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or

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antagonists and fragments and variants thereof.

Example 45: Cellular Adhesion Molecule (CAM) Expression on Endothelial Cells

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The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Briefly, endothelial cells (e.g., Human Umbilical Vein Endothelial cells (HUVECs)) are grown in a standard 96 well plate to confluence, growth medium is removed from the cells and replaced with 100 μl of 199 Medium (10% fetal bovine serum (FBS)). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10 μl volumes). Plates are then incubated at 37°C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100 μl of 0.1% paraformaldehyde-PBS(with Ca++ and Mg++) is added to each well. Plates are held at 4°C for 30 min. Fixative is removed from the wells and wells are washed 1X with PBS(+Ca,Mg) + 0.5% BSA and drained. 10 μl of diluted primary antibody is added to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10 μg/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed three times with PBS(+Ca,Mg) + 0.5% BSA. 20 μl of diluted ExtrAvidin-Alkaline

Phosphotase (1:5,000 dilution, referred to herein as the working dilution) are added to each well and incubated at 37°C for 30 min. Wells are washed three times with PBS(+Ca,Mg)+0.5% BSA. Dissolve I tablet of p-Nitrophenol Phosphate pNPP per 5 ml of glycine buffer (pH 10.4). 100 μ l of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphotase in glycine buffer: 1:5,000 (10°) > 10^{-0.5} > 10⁻¹ > 10^{-1.5}.5 μ l of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 μ l of pNNP reagent is then added to each of the standard wells. The plate is incubated at 37°C for 4h. A volume of 50 μ l of 3M NaOH is added to all wells. The plate is read on a plate reader at 405 nm using the background subtraction option on blank wells filled with glycine buffer only. Additionally, the template is set up to indicate the concentration of APconjugate in each standard well [5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

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Example 46: Alamar Blue Endothelial Cells Proliferation Assay

This assay may be used to quantitatively determine protein mediated inhibition of bFGF-induced proliferation of Bovine Lymphatic Endothelial Cells (LECs), Bovine Aortic Endothelial Cells (BAECs) or Human Microvascular Uterine Myometrial Cells (UTMECs). This assay incorporates a fluorometric growth indicator based on detection of metabolic activity. A standard Alamar Blue Proliferation Assay is prepared in EGM-2MV with 10 ng /ml of bFGF added as a source of endothelial cell stimulation. This assay may be used with a variety of endothelial cells with slight changes in growth medium and cell concentration. Dilutions of the protein batches to be tested are diluted as appropriate. Serum-free medium (GIBCO SFM) without bFGF is used as a non-stimulated control and Angiostatin or TSP-1 are included as a known inhibitory controls.

Briefly, LEC, BAECs or UTMECs are seeded in growth media at a density of 5000 to 2000 cells/well in a 96 well plate and placed at 37-C overnight. After the

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overnight incubation of the cells, the growth media is removed and replaced with GIBCO EC-SFM. The cells are treated with the appropriate dilutions of the protein of interest or control protein sample(s) (prepared in SFM) in triplicate wells with additional bFGF to a concentration of 10 ng/ml. Once the cells have been treated with the samples, the plate(s) is/are placed back in the 37° C incubator for three days. After three days 10 ml of stock alamar blue (Biosource Cat# DAL1100) is added to each well and the plate(s) is/are placed back in the 37°C incubator for four hours. The plate(s) are then read at 530nm excitation and 590nm emission using the CytoFluor fluorescence reader. Direct output is recorded in relative fluorescence units.

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Alamar blue is an oxidation-reduction indicator that both fluoresces and changes color in response to chemical reduction of growth medium resulting from cell growth. As cells grow in culture, innate metabolic activity results in a chemical reduction of the immediate surrounding environment. Reduction related to growth causes the indicator to change from oxidized (non-fluorescent blue) form to reduced (fluorescent red) form. i.e. stimulated proliferation will produce a stronger signal and inhibited proliferation will produce a weaker signal and the total signal is proportional to the total number of cells as well as their metabolic activity. The background level of activity is observed with the starvation medium alone. This is compared to the output observed from the positive control samples (bFGF in growth medium) and protein dilutions.

Example 47: Detection of Inhibition of a Mixed Lymphocyte Reaction

This assay can be used to detect and evaluate inhibition of a Mixed

Lymphocyte Reaction (MLR) by gene products (e.g., isolated polypeptides).

Inhibition of a MLR may be due to a direct effect on cell proliferation and viability, modulation of costimulatory molecules on interacting cells, modulation of adhesiveness between lymphocytes and accessory cells, or modulation of cytokine production by accessory cells. Multiple cells may be targeted by these polypeptides

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since the peripheral blood mononuclear fraction used in this assay includes T, B and natural killer lymphocytes, as well as monocytes and dendritic cells.

Polypeptides of interest found to inhibit the MLR may find application in diseases associated with lymphocyte and monocyte activation or proliferation. These include, but are not limited to, diseases such as asthma, arthritis, diabetes, inflammatory skin conditions, psoriasis, eczema, systemic lupus erythematosus, multiple sclerosis, glomerulonephritis, inflammatory bowel disease, crohn's disease, ulcerative colitis, arteriosclerosis, cirrhosis, graft vs. host disease, host vs. graft disease, hepatitis, leukemia and lymphoma.

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Briefly, PBMCs from human donors are purified by density gradient centrifugation using Lymphocyte Separation Medium (LSM®, density 1.0770 g/ml, Organon Teknika Corporation, West Chester, PA). PBMCs from two donors are adjusted to 2 x 10⁶ cells/ml in RPMI-1640 (Life Technologies, Grand Island, NY) supplemented with 10% FCS and 2 mM glutamine. PBMCs from a third donor is adjusted to 2 x 10⁵ cells/ml. Fifty microliters of PBMCs from each donor is added to wells of a 96-well round bottom microtiter plate. Dilutions of test materials (50 μl) is added in triplicate to microtiter wells. Test samples (of the protein of interest) are added for final dilution of 1:4; rhulL-2 (R&D Systems, Minneapolis, MN, catalog number 202-IL) is added to a final concentration of 1 μg/ml; anti-CD4 mAb (R&D Systems, clone 34930.11, catalog number MAB379) is added to a final concentration of 10 μg/ml. Cells are cultured for 7-8 days at 37°C in 5% CO₂, and 1 μC of [³H] thymidine is added to wells for the last 16 hrs of culture. Cells are harvested and thymidine incorporation determined using a Packard TopCount. Data is expressed as the mean and standard deviation of triplicate determinations.

Samples of the protein of interest are screened in separate experiments and compared to the negative control treatment, anti-CD4 mAb, which inhibits proliferation of lymphocytes and the positive control treatment, IL-2 (either as recombinant material or supernatant), which enhances proliferation of lymphocytes.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or

antagonists and fragments and variants thereof.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference. Further, the hard copy of the sequence listing submitted herewith and the corresponding computer readable form are both incorporated herein by reference in their entireties. Moreover, the hard copy of and the corresponding computer readable form of the Sequence Listing of Serial No. 60/124,270 are also incorporated herein by reference in their entireties.

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism refers on page121, line	ed to in the description N/A
B. IDENTIFICATIONOFDEPOSIT	Further deposits are identified on an additional sheet
Name of depositary institution American Type Culture College	ction
Address of depositary institution (including postal code and count 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	(אי
Date of deposit	Accession Number
20 May 1997	209059
C. ADDITIONAL INDICATIONS (leave blank if not applicable	r) This information is continued on an additional sheet
D. DESIGNATED STATES FOR WHICH INDICATION Europe In respect to those designations in which a European Participation will be made available until the publication or until the date on which application has been refused the issue of such a sample to an expert nominated by the	atent is sought a sample of the deposited on of the mention of the grant of the European patent or withdrawn or is deemed to be withdrawn, only by see person requesting the sample (Rule 28 (4) EPC).
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A. The indications made below relate to the microorganism referred to in the description		
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Page 2 ATCC Deposit No. 209061

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B.	IDENTIFI	CATIONOFDEPOSIT		Further deposits are identified on an additional sheet	
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C.	ADDITIO	NAL INDICATIONS (lea	ve blank if not applicabl	e) This information is continued on an additional sheet	
					
D.	DESIGNA	TED STATES FOR WH	ICH INDICATION	NS ARE MADE (if the indications are not for all designated States)	
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E.	SEPARAT	E FURNISHING OF INI	OICATIONS (leave h	lant il not applicable)	
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ATCC Deposit No. 209062

CANADA

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FINLAND

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UNITED KINGDOM

Page 2 ATCC Deposit No. 209062

DENMARK

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Applicant's or agent's file	-	International application I	
reference number	PA106PCT	approduction:	UNASSIGNED
			

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description				
on page, line, N/A				
B. IDENTIFICATIONOF DEPOSIT	Further deposits are identified on an additional sheet			
Name of depositary institution American Type Culture Colle	ction			
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Address of depositary institution (including postal code and count 10801 University Boulevard	מי)			
Manassas, Virginia 20110-2209				
United States of America				
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Date of deposit 20 May 1997	Accession Number			
	209063			
C. ADDITIONAL INDICATIONS (leave blank if not applicable	r) This information is continued on an additional sheet			
D. DESIGNATED STATES FOR WHICH INDICATION	S ARE MADE (if the indications are not for all designated States)			
Europe				
In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent				
or until the date on which application has been refused or withdrawn or is, deemed to be withdrawn, only by				
the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).				
E. SEPARATE FURNISHING OF INDICATIONS (leave bi	ank if not applicable)			
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ATCC Deposit No. 209063

CANADA

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UNITED KINGDOM

Page 2 ATCC Deposit No. 209063

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Applicant's or agent's file reference number	PA106PCT	International application	UNASSIGNED

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism refers on page	ed to in the description N/A .		
B. IDENTIFICATIONOFDEPOSIT	Further deposits are identified on an additional sheet		
Name of depositary institution American Type Culture Colle	ction		
Address of depositary institution (including postal code and count 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	ry)		
Date of deposit	Accession Number		
20 May 1997	209064		
C. ADDITIONAL INDICATIONS (leave blank if not applicable	r) This information is continued on an additional sheet		
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States) Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).			
E. SEPARATE FURNISHING OF INDICATIONS (leave b	lank if not applicable)		
The indications listed below will be submitted to the Internation Number of Deposit")	nal Bureau later (specify the general nature of the indications e.g., "Accession		
For receiving Office use only	For International Bureau use only		
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ATCC Deposit No. 209064

CANADA

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NORWAY

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UNITED KINGDOM

Page 2 ATCC Deposit No. 209064

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Applicant's or agent's file reference number	PA106PCT	International application'	UNASSIGNED

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

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A	The indications made on page	below relate to the mici	roorganism referr	ed to in the description N/A
В.	IDENTIFICATION	OFDEPOSIT		Further deposits are identified on an additional sheet
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Da	te of deposit		<u> </u>	Accession Number
	2	20 May 1997		209065
C.	ADDITIONAL IND	OICATIONS (leave blo	ank if not applicable	This information is continued on an additional sheet
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ATCC Deposit No. 209065

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UNITED KINGDOM

Page 2 ATCC Deposit No. 209065

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NETHERLANDS

Applicant's or agent's file reference number	PA106PCT	International application	UNASSIGNED

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism refer	red to in the description	
on page 121 , line	N/A .	
B. IDENTIFICATIONOF DEPOSIT	Further deposits are identified on an additional sheet	
Name of depositary institution American Type Culture Colle	ction	
Address of depositary institution (including postal code and count 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	ry)	
Date of deposit	Accession Number	
20 May 1997	209066	
C. ADDITIONAL INDICATIONS (leave blank if not applicable	e) This information is continued on an additional sheet	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States) Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).		
E. SEPARATE FURNISHING OF INDICATIONS (leave b	lank if not applicable)	
The indications listed below will be submitted to the Internation Number of Deposit")	al Bureau later (specify the general nature of the indications e.g., "Accession .	
For receiving Office use only	For International Bureau use only	
This sheet was received with the international application	This sheet was received by the International Bureau on:	
Authorities and Processing Div. (703) 305-3639	Authorized officer	

WO 00/55350 PCT/US00/05882

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ATCC Deposit No. 209066

CANADA

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UNITED KINGDOM

Page 2 ATCC Deposit No. 209066

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NETHERLANDS

Applicant's or agent's file reference number	PA106PCT	International application?	UNASSIGNED

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page		
B. IDENTIFICATIONOFDEPOSIT	Further deposits are identified on an additional sheet	
Name of depositary institution American Type Culture Colle	ection	
Address of depositary institution (including postal code and count 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	iry)	
Date of deposit	Accession Number	
20 May 1997	209067	
C. ADDITIONAL INDICATIONS (leave blank if not applicable	e) This information is continued on an additional sheet	
D. DESIGNATED STATES FOR WHICH INDICATION Europe In respect to those designations in which a European P microorganism will be made available until the publication or until the date on which application has been refused the issue of such a sample to an expert nominated by the	atent is sought a sample of the deposited on of the mention of the grant of the European patent or withdrawn or is deemed to be withdrawn, only by ne person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave b	· ·	
The indications listed below will be submitted to the Internation Number of Deposit")		
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Authorized officer Processing Div. (703) 303-3039	Authorized officer	

ATCC Deposit No. 209067

CANADA

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Page 2 ATCC Deposit No. 209067

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A. The indications made below relate to the microorganism refer	red to in the description
on page	N/A
B. IDENTIFICATIONOFDEPOSIT	Further deposits are identified on an additional sheet
Name of deposite win single American Trans Culture Culture	
Name of depositary institution American Type Culture Colle	ction
Address of depositary institution (including postal code and count	n)
10801 University Boulevard Manassas, Virginia 20110-2209	
United States of America	
Date of deposit	Accession Number
20 May 1997	209068
C. ADDITIONAL INDICATIONS (leave blank if not applicable	This information is continued on an additional sheet
D. DESIGNATED STATES FOR WHICH INDICATION	S ARE MADE (if the indications are not for all designated States)
Europe In respect to those designations in which a European Pamicroorganism will be made available until the publication or until the date on which application has been refused the issue of such a sample to an expert nominated by the	on of the mention of the grant of the European patent
E. SEPARATE FURNISHING OF INDICATIONS (leave b)	ank if not applicable)
The indications listed below will be submitted to the Internation Number of Deposit")	al Bureau later (specify the general nature of the indications e.g., "Accession
For receiving Office use only	For International Bureau use only
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Authorizedofficer	Authorized officer
POT/Internal/1/pp1 Processing Div. (703) 365-6639	

ATCC Deposit No. 209068

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

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FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

Page 2 ATCC Deposit No. 209068

DENMARK

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SWEDEN

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NETHERLANDS

Applicant's or agent's file reference number PA106PCT International application!	INASSIGNED

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European pate or until the date on which application has been refused or withdrawn or is, deemed to be withdrawn only by				
Name of depositary institution American Type Culture Collection Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America Date of deposit 20 May 1997 209069 C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States) Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European pate or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	404			
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WO 00/55350 PCT/US00/05882

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ATCC Deposit No. 209069

CANADA

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UNITED KINGDOM

Page 2 ATCC Deposit No. 209069

DENMARK

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SWEDEN

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NETHERLANDS

Applicant's or agent's file reference number	PA106PCT	International application	UNASSIGNED

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page 121 , line N/A				
B. IDENTIFICATIONOF DEPOSIT	Further deposits are identified on an additional sheet			
Name of depositary institution American Type Culture Collection				
Address of depositary institution (including postal code and count 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	(v)			
Date of deposit Accession Number				
12 January 1998	209579			
C. ADDITIONAL INDICATIONS (leave blank if not applicable	r) This information is continued on an additional sheet			
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States) Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).				
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)				
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(703) 305-3639				

ATCC Deposit No. 209579

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

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UNITED KINGDOM

Page 2 ATCC Deposit No. 209579

DENMARK

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SWEDEN

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NETHERLANDS

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Applicant's or agent's file reference number	PA106PCT	International application	UNASSIGNED

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism refer on page121, line	red to in the description N/A
B. IDENTIFICATIONOFDEPOSIT	Further deposits are identified on an additional sheet
Name of depositary institution American Type Culture Colle	
Address of depositary institution (including postal code and coun	iry)
10801 University Boulevard Manassas, Virginia 20110-2209	
United States of America	
Date of deposit	Accession Number
12 January 1998	209578
C. ADDITIONAL INDICATIONS (leave blank if not applicable	le) This information is continued on an additional sheet
D. DESIGNATED STATES FOR WHICH INDICATION	NS ARE MADE (if the indications are not for all designated States)
Europe In respect to those designations in which a European P microorganism will be made available until the publicati or until the date on which application has been refused the issue of such a sample to an expert nominated by the	on of the mention of the grant of the European natent
E. SEPARATE FURNISHING OF INDICATIONS (leave)	blank if not applicable)
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ATCC Deposit No. 209578

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

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UNITED KINGDOM

Page 2 ATCC Deposit No. 209578

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NETHERLANDS

Applicant's or agent's file		International application	
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism refer on page121 , line	red to in the description N/A
B. IDENTIFICATIONOF DEPOSIT	Further deposits are identified on an additional sheet
Name of depositary institution American Type Culture Colle	
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Address of depositary institution (including postal code and count	ry)
10801 University Boulevard Manassas, Virginia 20110-2209	
United States of America	
Date of deposit	Accession Number
16 July 1998	203067
C. ADDITIONAL INDICATIONS (leave blank if not applicable	This information is continued on an additional sheet
D. DESIGNATED STATES FOR WHICH INDICATION	IS ARE MADE (if the indications are not for all designated States)
Europe In respect to those designations in which a European Pamicroorganism will be made available until the publication or until the date on which application has been refused the issue of such a sample to an expert nominated by the	on of the mention of the grant of the European patent
E. SEPARATE FURNISHING OF INDICATIONS (leave b)	lank if not applicable)
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Form PCT/RO/134 (July 1992)

ATCC Deposit No. 203067

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

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UNITED KINGDOM

Page 2 ATCC Deposit No. 203067

DENMARK

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NETHERLANDS

Applicant's or agent's file reference number	PA106PCT	International application	UNASSIGNED

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

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		16 July 1998		203068
C.	ADDITIONA	L INDICATIONS (leav	e blank if not applicab	le) This information is continued on an additional sheet
				
D.	DESIGNATE	D STATES FOR WHI	CH INDICATIO	NS ARE MADE (if the indications are not for all designated States)
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Form PCT/RO/134 (July 1992)

ATCC Deposit No. 203068

CANADA

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UNITED KINGDOM

Page 2 ATCC Deposit No. 203068

DENMARK

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SWEDEN

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Applicant's or agent's file PA10CDCT International application				
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism refers	red to in the description N/A
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B. IDENTIFICATIONOFDEPOSIT	Further deposits are identified on an additional sheet
Name of depositary institution American Type Culture College	ction
Address of depositary institution (including postal code and count 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	ny)
Dateofdeposit	Accession Number
1 February 1999	203609
C. ADDITIONAL INDICATIONS (leave blank if not applicable	This information is continued on an additional sheet
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Authorized officer PCT/Internat Appl Processing Div. (703) 305-3839	Authorized officer

Form PCT/RO/134 (July 1992)

ATCC Deposit No. 203609

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

Page 2 ATCC Deposit No. 203609

DENMARK

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Applicant's or agent's file reference number	PA106PCT	International application	UNASSIGNED

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

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ATCC Deposit No. 203610

CANADA

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UNITED KINGDOM

Page 2 ATCC Deposit No. 203610

DENMARK

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reference number PA106PCT International application UNASSIGNED	Applicant's or agent's file reference number	PA106PCT	International application	UNASSIGNED

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit") For receiving Office use only This sheet was received by the International Bureau on: Authorized officer Authorized officer			
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	(703) 305-3839		

Form PCT/RO/134 (July 1992)

PCT/US00/05882

ATCC Deposit No. 203485

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement; or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

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FINLAND

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UNITED KINGDOM

Page 2 ATCC Deposit No. 203485

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NETHERLANDS

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Applicant's or agent's file reference number	PA106PCT	International application	UNASSIGNED

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

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A. The indications made below relate to the microorganism refers	red to in the description N/A	
B. IDENTIFICATION OF DEPOSIT	Further deposits are identified on an additional sheet	
Name of depositary institution American Type Culture Colle	ction	
Address of depositary institution (including postal code and count 10801 University Boulevard	ייי)	
Manassas, Virginia 20110-2209		
United States of America		
Date of deposit	Accession Number	
18 June 1999	PTA-252	
C. ADDITIONAL INDICATIONS (leave blank if not applicable	e) This information is continued on an additional sheet	
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D. DESIGNATED STATES FOR WHICH INDICATION	S ARE MADE (if the indications are not for all designated States)	
Europe		
In respect to those designations in which a European Pr	atent is sought a sample of the deposited	
microorganism will be made available until the publication or until the date on which application has been refused or until the date on which application has	or withdrawn or is deemed to be withdrawn, only by	
the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).		
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ATCC Deposit No. PTA-252

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

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UNITED KINGDOM

Page 2 ATCC Deposit No. PTA-252

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Applicant's or agent's file reference number	PA106PCT	International application '	UNASSIGNED

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description		
onpage 121 , line	N/A	
B. IDENTIFICATIONOFDEPOSIT	Further deposits are identified on an additional sheet	
Name of depositary institution American Type Culture Colle	ction	
Address of depositary institution (including postal code and count	(ימ	
10801 University Boulevard Manassas, Virginia 20110-2209		
United States of America		
Date of deposit	Accession Number	
18 June 1999	PTA-253	
C. ADDITIONAL INDICATIONS (leave blank if not applicable	e) This information is continued on an additional sheet	
D. DESIGNATED STATES FOR WHICH INDICATION	VS ARE MADE (if the indications are not for all designated States)	
Europe		
In respect to those designations in which a European Pamicroorganism will be made available until the publication	atent is sought a sample of the deposited	
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E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)		
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Authorized officer Appl Processing Div. (703) 305-3639	Authorized officer	
(100) 200-3033 . 7		

Form PCT/RO/134 (July 1992)

ATCC Deposit No. PTA-253

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

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Page 2 ATCC Deposit No. PTA-253

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	599		
Applicant's or agent's file reference number	PA106PCT	International application	UNASSIGNED

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism refers on page	red to in the description N/A	
B. IDENTIFICATIONOF DEPOSIT	Further deposits are identified on an additional sheet	
Name of depositary institution American Type Culture College	ction	
Address of depositary institution (including postal code and count 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	(אָר	
Date of deposit	Accession Number	
22 December 1999	PTA-1081	
C. ADDITIONAL INDICATIONS (leave blank if not applicable	This information is continued on an additional sheet	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States) Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent		
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ATCC Deposit No. PTA-1081

CANADA

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Page 2 ATCC Deposit No. PTA-1081

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What Is Claimed Is:

- 1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:
- (a) a polynucleotide fragment of SEQ ID NO:X or a polynucleotide fragment of the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X;
- (b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:Y or a polypeptide fragment encoded by the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X;
 - (c) a polynucleotide encoding a polypeptide fragment of a polypeptide encoded by SEQ ID NO:X or a polypeptide fragment encoded by the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X;
- (d) a polynucleotide encoding a polypeptide domain of SEQ ID NO:Y or a polypeptide domain encoded by the cDNA sequence included in the related cDNA clone, which is hybridizable to SEO ID NO:X:
 - (e) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:Y or a polypeptide epitope encoded by the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X;
 - (f) a polynucleotide encoding a polypeptide of SEQ ID NO:Y or the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X, having biological activity;
 - (g) a polynucleotide which is a variant of SEQ ID NO:X;
 - (h) a polynucleotide which is an allelic variant of SEQ ID NO:X;
 - (i) a polynucleotide which encodes a species homologue of the SEQ ID NO:Y;
- (j) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide

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sequence of only A residues or of only T residues.

- 2. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a protein.
- 3. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:Y or the polypeptide encoded by the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X.

4. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:X or the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X.

- 5. The isolated nucleic acid molecule of claim 2, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.
- 20 6. The isolated nucleic acid molecule of claim 3, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.
- 7. A recombinant vector comprising the isolated nucleic acid molecule of claim 1.
 - 8. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 1.
- 30 9. A recombinant host cell produced by the method of claim 8.

- 10. The recombinant host cell of claim 9 comprising vector sequences.
- 11. An isolated polypeptide comprising an amino acid sequence at least 5 95% identical to a sequence selected from the group consisting of:
 - (a) a polypeptide fragment of SEQ ID NO:Y or of the sequence encoded by the cDNA included in the related cDNA clone;
 - (b) a polypeptide fragment of SEQ ID NO:Y or of the sequence encoded by the cDNA included in the related cDNA clone, having biological activity;
- (c) a polypeptide domain of SEQ ID NO:Y or of the sequence encoded by the cDNA included in the related cDNA clone;
 - (d) a polypeptide epitope of SEQ ID NO:Y or of the sequence encoded by the cDNA included in the related cDNA clone;
- (e) a full length protein of SEQ ID NO:Y or of the sequence encoded by the cDNA included in the related cDNA clone;
 - (f) a variant of SEQ ID NO:Y;
 - (g) an allelic variant of SEQ ID NO:Y; or
 - (h) a species homologue of the SEQ ID NO:Y.
- 20 12. The isolated polypeptide of claim 11, wherein the full length protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.
- 13. An isolated antibody that binds specifically to the isolated polypeptide 25 of claim 11.
 - 14. A recombinant host cell that expresses the isolated polypeptide of claim 11.
- 30 15. A method of making an isolated polypeptide comprising:

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- (a) culturing the recombinant host cell of claim 14 under conditions such that said polypeptide is expressed; and
 - (b) recovering said polypeptide.
- 5 16. The polypeptide produced by claim 15.
 - 17. A method for preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of the polypeptide of claim 11 or the polynucleotide of claim 1.

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- 18. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:
- (a) determining the presence or absence of a mutation in the polynucleotide of claim 1; and
- (b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.
 - 19. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:
 - (a) determining the presence or amount of expression of the polypeptide of claim 11 in a biological sample; and
 - (b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.
- 25 20. A method for identifying a binding partner to the polypeptide of claim 11 comprising:
 - (a) contacting the polypeptide of claim 11 with a binding partner; and
 - (b) determining whether the binding partner effects an activity of the polypeptide.

- 21. The gene corresponding to the cDNA sequence of SEQ ID NO:Y. .
- 22. A method of identifying an activity in a biological assay, wherein the method comprises:
- 5 (a) expressing SEQ ID NO:X in a cell;
 - (b) isolating the supernatant;
 - (c) detecting an activity in a biological assay; and
 - (d) identifying the protein in the supernatant having the activity.
- The product produced by the method of claim 20.

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       Steve Ruben
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<213> Homo sapiens

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<213> Homo sapiens

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<222> (1517)
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<222> (2110)
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<213> Homo sapiens
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<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (884)
<223> n equals a,t,g, or c
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<222> (1019)
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<222> (507)
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<222> (556)
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<222> (501)

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<222> (44)

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31

PCT/US00/05882

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32

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33

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34

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35

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<211> 563
<212> DNA
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<223> n equals a,t,g, or c
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gaggetgtgc tgaagetgte tetegggtee teaataaget tggaggagtt aagtatgaca 180
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<212> DNA
<213> Homo sapiens
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<220>

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<211> 920

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<213> Homo sapiens
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<221> misc feature
<222> (572)
<223> n equals a,t,g, or c
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<221> misc feature
<222> (573)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (618)
<223> n equals a,t,g, or c
<400> 52
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<222> (621)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (725)
<223> n equals a,t,g, or c
<400> 53
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<211> 1090
<212> DNA
<213> Homo sapiens
<220>
<221> misc feature
<222> (1024)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (1034)
<223> n equals a,t,g, or c
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<400> 54

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<213> Homo sapiens
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<222> (766)
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<211> 985
<212> DNA
<213> Homo sapiens
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<223> n equals a,t,g, or c
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<223> n equals a,t,g, or c
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<221> misc feature
<222> (973)
<223> n equals a,t,g, or c
<400> 56
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<212> DNA
<213> Homo sapiens
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<223> n equals a,t,g, or c

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<212> DNA

<213> Homo sapiens

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<222> (1081)
<223> n equals a,t,g, or c
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<221> misc feature
<222> (1090)
<223> n equals a,t,g, or c
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59

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<222> (3)
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<211> 2009
<212> DNA
<213> Homo sapiens
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<222> (1955)
<223> n equals a,t,g, or c
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<221> misc feature
<222> (1959)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (2008)
<223> n equals a,t,g, or c
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gtgctggaat gacagaagag gcacaacgac tctgtaaacg ctgtggtcaa gcatggagag 180
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<223> n equals a,t,g, or c
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<222> (526)
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<211> 4302
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<213> Homo sapiens
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<220>
<221> misc feature
<222> (4270)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (4274)
<223> n equals a,t,g, or c
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 <221> misc feature
 <222> (4296)
<223> n equals a,t,g, or c
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77

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2328

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<222> (119)

<220>

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<223> n equals a,t,g, or c

<221> misc feature

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184

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189

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<220>
<221> misc feature
<222> (897)
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ggtttgtgtg tttctgtttt gtttctctcc ccctgcaggg ctgtttkcgg ggtggggtgg 180
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<223> n equals a,t,g, or c
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<222> (244)
<223> n equals a,t,g, or c
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<213> Homo sapiens
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206

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214B
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<210> 248

<211> 2225

<212> DNA

<213> Homo sapiens

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<223> n equals a,t,g, or c
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<221> misc feature
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214

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226

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230

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235

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236

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PCT/US00/05882

261

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<223> n equals a,t,g, or c
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<223> n equals a,t,g, or c
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<223> n equals a,t,q, or c
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<222> (2098)
<223> n equals a,t,g, or c
<220>
<221> misc feature
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<222> (2114)
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<221> misc feature
<222> (2117)
<223> n equals a,t,g, or c
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<223> n equals a,t,g, or c

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<222> (150)
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<223> n equals a,t,g, or c
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<213> Homo sapiens
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<212> DNA
<213> Homo sapiens
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<221> misc feature
<222> (521) ·
<223> n equals a,t,g, or c
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<222> (525)
<223> n equals a,t,g, or c
<220>
<221> misc feature
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<210> 361
<211> 1680
<212> DNA
<213> Homo sapiens
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<221> misc feature
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<223> n equals a,t,g, or c
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302

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<212> DNA

<213> Homo sapiens

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<222> (136)

<221> misc feature

<223> n equals a,t,g, or c

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<220>

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<211> 1267
<212> DNA
<213> Homo sapiens
<220>
<221> misc feature
<222> (4)
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<223> n equals a,t,g, or c
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<221> misc feature
<222> (214)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (1165)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (1255)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (1262)
<223> n equals a,t,g, or c
<400> 380
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atgtatatgg ctttactcaa gcaratctca tctcatgaca ggcagccacg tctcaacatg 180
ggtaaggggt gggggtggag gggaatgtgt gcancgtttt tacctaggca ccatcattta 240
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gtcaagattt tacttggcat tgagtagttt ttttcaatag taggtaattc cttagagata 360
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ataaccatga atacacttac agttaggatt tgtggtaagg tacctctcaa cattaccaaa 600
atcatttctt tagagggaag gaataatcat tcaaatgaac tttaaaaaag caaatttcat 660
gcactgatta aaataggatt attttaarta caaaaggcat tttatatgaa ttataaactg 720
aagagettaa agatagttae aaaatacaaa agtteaacet ettacaataa getaaacgea 780
atgtcatttt taaaaagaag gacttagggt gtcgttttca catatgacaa tgttgcattt 840
atgatgcagt ttcaagtacc aaaacgttga attgatgatg cagttttcat atatcgagat 900
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<211> 1031
<212> DNA
<213> Homo sapiens
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<221> misc feature
<222> (1015)
<223> n equals a,t,g, or c
<400> 381
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caaaaatgtt tcacttccta acagttttcc tttttccact gtgtgactga aagctcctat 180
atcattttat atttctgaat ctataaaaca aaacaaacaa gcctgamagt gtctggarga 240
recaaaggtg geeteeetgt eeccaaatat attggetata tgagagtaat tttaceeete 300
tacgtaccta aaggcaccca gttcactagt ctgtggggtc ctggagcctg tctcttcttt 360
ctggaggttc aaactgaata gcaataatta cgttacccaa agcatgtgga ggaaaagtga 420
aaccagccac ggagacgctg gcccacgggc teggeetgeg gtgtggeetg ctttgetcac 480
cagogtcago ogotcattto ottotoatga agtoccatot ggtcatgggg acgagggccg 540
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catgcatgca aagtcaaagt ttaaaatttt atccttttca aatagatgat ataatatacc 660
tatacatgat ataatatttg tatatatgaa atctctctat atttgtttaw tttgagccat 720
tcaatctaaa ccaatgtaca ggtgtacaat gaaaaattta aatgcttagt tatttttccc 780
aacacagtgt aaagtcaccc teetetgaga gtgggatgtg cagagttttg atgttgeage 840
tttgctcact tcctggcaag ggcaggtcat gcctcaattt gtaatgggag tctggggtaa 900
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gggggggccc c
<210> 382
<211> 1597
<212> DNA
<213> Homo sapiens
<220>
<221> misc feature
<222> (1577)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (1579)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (1597)
<223> n equals a,t,g, or c
<400> 382
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gataggcgac acgccgrcgg gcggctgagg cgggaatggc tgctgtactg cagcgcgtcg 120
ageggetgte caategagte gtgegtgtgt tgggetgtaa eeegggteee atgaeeetee 180
aaggcaccaa cacctaccta gtggggaccg gccccaggag aatcctcatt gacactggag 240
aaccagcaat tccagaatac atcagctgtt taaagcaggc tctaactgaa tttaacacag 300
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caatccagga aattgtagtg actcactggc accgagatca ttctggaggc ataggagata 360
tttgtaaaag catcaataat gacactacct attgcattaa aaaactccca cggaatcctc 420
agagagaaga aattatagga aatggagagc aacaatatgt ttatctgaaa gatggagatg 480
tgattaagac tgagggagcc actctaagag ttctatatac ccctggccac actgatgatc 540
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<210> 383
<211> 175
<212> DNA
<213> Homo sapiens
<220>
<221> misc feature
<222> (95)
<223> n equals a,t,g, or c
<400> 383
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ccaaacatct actacaaggt atgagggctc ctctnacgtg gctatcctga atccagccct 120
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<210> 384
<211> 2171
<212> DNA
<213> Homo sapiens
<220>
<221> misc feature
<222> (2166)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (2170)
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<223> n equals a,t,g, or c
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<400> 384
agaacaagag ctggacacat taaaaagaaa gagtccatca gatttgtgga aagaagactt 60
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cggacttcct gggaaagtgg ggaaggccaa ggggaaaaaa acacaaatgg ctgaagtttt 180
gccttctccg cgtggtcaaa gagtcattcc acgaataacc atagaaatga aagcagaggc 240
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tggtgtggaa ctagaaggcc taaaacaaag attagaaaag aaacagaaaa gagaaccagg 360
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<210> 385

<211> 2364

<212> DNA

<213> Homo sapiens

<220>

<221> misc feature

<222> (19)

<223> n equals a,t,g, or c

<400> 385

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323

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<210> 387

<211> 2683

<212> DNA

<213> Homo sapiens

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<221> misc feature
<222> (40)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (2649)
<223> n equals a,t,g, or c
<400> 387
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tttcctagat ccacactttc aaagagaaac ccctccagaa ctcccacct gacagcccaa 180
caccaccttc ctcctggctt ccagggggca gcccagtgga atggaaagaa tgtgggattt 240
ggagtcagac aagcctgagt ccagttcccc gtttagaact cattagctgt gtgactctgg 300
gtgagtccct taacccctct gagcccgggt ctcttcatta gttgaaaggg atagtaatac 360
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tggtagctct tgttgcttcc cgttcagcgt cacatctgca gtggagcctg aaaaggctcc 480
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ccctgggatc tcaggagggc agccacggag ggggaggccc cagatgcgct gtgccaaagc 660
caggiccgag gccaaagttc tccctgccat ccttggtgcc gtcctgcccc ttcctccttc 720
atgeetggge etgeaggeae eccageeace actgagteea eteggagtge eetgtgttee 780
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ccaaccactc atecetettt ttettette caccactece caccccaget gtagttaatt 1140
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gttttggatg gagttaaact tgatgccagt gggcagtgca tgtggaaagt atcagagtaa 2340
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                                                                   2683
<210> 388
<211> 1446
<212> DNA
<213> Homo sapiens
<220>
<221> misc feature
<222> (35)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (37)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (57)
<223> n equals a,t,g, or c
<400> 388
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gaattcccgg gtcgacccac gcgtccgaar argaggtgga ggargagggt gatgttgata 120
gtgatgaaga agaggaggaa gatgaggaga gctcctcgga gggcttggag gctgaggact 180
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cccagtgccc tactctgcat ttcctggaag gtggggagga ctctgattca gacagtgagg 300
aagaggacga tgaggaagag gatgatgaag atgaagacga cgatgatgat gaggaggatg 360
gtgatgaggt gcctgtaccc agctttgggg aggccatggc ttactttgcc atggtcaaga 420
ggtacctgac ctccttcccc attgatgacc gcgtgcagag ccacatcctc cacttggaac 480
acgatctggt tcatgtgacc aggaagaacc acgccaggca ggcgggagtt cgaggtcttg 540
gacatcaaag ctgagtcact ggacctagct gtgcccccaa cctagattgg cagcaccacc 600
ccagggcaga ggactctctg ggcacccgct gtgcatggag ccagagtgca gagccccaga 660
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gctgaggtca gcctcactgc ctgcttattg cctctttctc agaatcctct ttcctcccca 780
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cggagagtgc ctctcccctc tgccatccac gtcaggtctt tggtgggggg accccaaagc 960
cattetggga agggetecag aagaaggtee ageetaggee eeetgeaagg etggeageee 1020
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cotgettetg cotgetttee acctececag tecetttete tggecetgte catgtgaett 1140
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ccaagggege tteettgtgg geagetgeag geeceatgee teteeteect etetggeagg 1260
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tttcccagtt tgatttcaat aaatctgtcc actccccttt tgtgggggtg aacgttttaa 1380
садссавава алававава вазававава валавала вазавалава дазававава 1440
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aaaaaa
                                                                   1446
<210> 389
<211> 723
<212> DNA
<213> Homo sapiens
<220>
<221> misc feature
<222> (705)
<223> n equals a,t,g, or c
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ataaaaactg acttgtaatc caggctatgt ctctttttag cttcgtaatc tttggcaagg 180
ccattggatt cttcagctgt acaattagga gactcgatca ggtgattgcc tttctcagct 240
gtcagttctc taatttcagg cttggtagct tgtaggaact gaaattgcaa ttaaaacctt 300
tataaactca aactaaatca tgaattacag aaaaagtcca ttcttccaaa acttgatgtt 360
accacactta caagtttaaa atatgaagtc gactgtttaa aggattctgc atatattcta 420
gtgtgcacat tcagaaacat ttttcttgga aaaagtaccc aacatttttt ataactgcac 480
atattaattt attgccagaa taaattgcat tgcatgctaa ataaagtcag ataattcaaa 540
tccatttgct tttatgtagt ttttcttcta aatgtcaaca ttttggaatt aaaatgttta 600
tggttttata tgagggtagg aaatcttaac tgctttgggg ggtattgttt ataggctttt 660
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ggg
                                                                   723
<210> 390
<211> 1046
<212> DNA
<213> Homo sapiens
<400> 390
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gaaccagcca gatgttegge eccageeeee ttegeeeega gagggeeete tgeetgetge 120
ccgacctgct ggtgccactc tggaaagggc caagactctc tccccaggga agaatggggt 180
cgtcaaagac gtttttgcct ttgggggtgc cgtggagaac cccgagtact tgacacccca 240
gggaggaget geceeteage eccaecetee teetgeette ageceageet tegacaacet 300
ctattactgg gaccaggacc caccagagcg gggggctcca cccagcacct tcaaagggac 360
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aatggtgtca gtatccaggc tttgtacaga gtgcttttct gtttagtttt tactttttt 900
gttttgtttt tttaaagatg aaataaagac ccagggggag aatgggtgtt gtatggggag 960
gcaagtgtgg ggggtccttc tccacaccca ctttgtccat ttgcaaatat attttggaaa 1020
acaaaaaaa aaaaaaaaa aaaaaa
                                                                  1046
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<211> 699
<212> DNA
<213> Homo sapiens
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atttggtgtg ctgttgaagg ggggagacta gagaaatggc agggaacctc ttatccgggg 180
caggtaggcg cctgtgggac tgggtgcctc tggcgtgcag aagcttctct cttggtgtgc 240
ctagattgat cggtataagg ctcactctcc cgccccccaa agtggttgat cgttggaacg 300
agaaaagggc catgttcgga gtgtatgaca acatcgggat cctgggaaac tttgaaaagc 360
accccaaaga actgatcagg gggcccatat ggcttcgagg ttggaaaggg aatgaattgc 420
aacgttgtat ccgaaagagg aaaatggttg gaagtagaat gttcgctgat gacctgcaca 480
accttaataa acgcatccgc tatctctaca aacactttaa ccgacatggg aagtttcgat 540
agaagagaaa gctgagaact tcggaaaagg ctcatctgtc accctggaga agggaaactg 600
tacttttccc tgtgaggaaa cggctttgta ttttctctgt aataaaatgg ggcttctttg 660
gaaaaaaaa aaaaaaaaaa aaaaaaaaaa aagtcgacc
<210> 392
<211> 1545
<212> DNA
<213> Homo sapiens
<220>
<221> misc feature
<222> (24)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (25)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (54)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (58)
<223> n equals a,t,g, or c
<400> 392
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ccggactcgg acgcgtggta gccccaggat gggtgagttc aacgagaaga agacaacatg 120
tggcaccgtt tgcctcaagt acctgctgtt tacctacaat tgctgcttct ggctggctgg 180
cctggctgtc atggcagtgg gcatctggac gctggccctc aagagtgact acatcagcct 240
gctggcctca ggcacctacc tggccacagc ctacatcctg gtggtggcgg gcactgtcgt 300
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328

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cetgtacttc atcetgetce teatcatett tetgetggag atcategetg gtateetege 420
ctacgcctac taccagcagc tgaacacgga gctcaaggag aacctgaagg acaccatgac 480
caagegetae caccageegg gecatgagge tgtgaceage getgtggace agetgeagea 540
ggagttccac tgctgtggca gcaacaactc acaggactgg cgagacagtg agtggatccg 600
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gttggagacc ttcatccagg agcacctgag ggtcattggg gctgtggggga tcggcattgc 780
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<210> 393
<211> 749
<212> DNA
<213> Homo sapiens
<220>
<221> misc feature
<222> (490)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (748)
<223> n equals a,t,g, or c
<400> 393
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<211> 611
<212> DNA
<213> Homo sapiens
<400> 394
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agccggaaga gcgtttccca aagtgtattc tgcggaacta gcacctactg tgttctcaac 180
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tccttttaaa a
<210> 395
<211> 1856
<212> DNA
<213> Homo sapiens
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<221> misc feature
<222> (1851)
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gagccccggc cggccaggcc ctgccgctca tggtgccagc ccagagaggg gccagcccgg 180
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330

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<210> 396
<211> 2651
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<213> Homo sapiens
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<221> misc feature
<222> (45)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (47)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (2642)
<223> n equals a,t,g, or c
<400> 396
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aaaggaaagt ttcaggacaa ttttgaattc gttcagtggt tcaagaagtt tttcgatgca 480
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cggaattett cactecaaat catgtgetta actgtaaaat actecetttt gttateetta 1020
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gtttcactgc attgtatatt ttttcatttg gtacacaaag aatgtattct tcataggttt 1320
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<211> 1750
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<213> Homo sapiens
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<222> (2)
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<223> n equals a,t,g, or c

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<222> (70)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (107)
<223> n equals a,t,g, or c
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<213> Homo sapiens
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<222> (150)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (1330)
<223> n equals a,t,g, or c
<400> 404
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gagagtgaaa ttatgtgata cactgaaatn acttttgttt ttcttctaac tcatacaaaa 180
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tgtgtctgtg tgccctcctg ggagtcagtc agcgctcagg ccaggactgt gcaggqccag 780
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<210> 405
<211> 482
<212> DNA
<213> Homo sapiens
<220>
<221> misc feature
<222> (440)
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<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (469)
<223> n equals a,t,g, or c
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cacctaccca caaactagtg gatgataaat tttggctatt cagaagacgt ttattatagg 180
agtatgtaga ttttccatag agtgctgtta tgtgacttga attttagtct cggccctgcc 240
tctgacattg tcggtggttt atcctggttc caggaaataa gactagcctt ttcctcatga 300
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ctgctctaca ccagtgaata atttacactc taataggggg tggtaactat aaagatgata 420
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<210> 406
<211> 1413
<212> DNA
<213> Homo sapiens
<220>
<221> misc feature
<222> (9)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (24)
<223> n equals a,t,g, or c
<400> 406
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ttatttctgt tcggggccca tctatcctgg acacggcatg atgttcgtcc gcaacgattg 180
caaggtgttc agattttgca aatctaaatg tcataaaaac tttaaaaaaga agcgcaatcc 240
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taattcattt gaatttgaaa aacgtagaaa tgaacctatc aaataccagc gagagctatg 360
gaataaaact attgatgcga tgaagagat tgaagaaatc aaacagaagc gccaagctaa 420
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aactaatggc aaaaattcat ggctagtgat gtataaaata aaatattctt tgcagtaaaa 960
tattcccttt gttaatgtta tagaaggggg gatacaaaaa ggaactaaca atttgtatgg 1020
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341

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cagtgtcaga tatttttatt ttagtatttc ctgttttggt ttatttgcat cttagaagag 1080
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<210> 407
<211> 1693
<212> DNA
<213> Homo sapiens
<220>
<221> misc feature
<222> (1548)
<223> n equals a,t,g, or c
<400> 407
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gageteegee eggaggtaet gtgaggeegt tagagetgge ggtggatgae tteegeatte 480
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tcgagacagt tct
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<210> 408 <211> 1342

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<212> DNA
<213> Homo sapiens
<220>
<221> misc feature
<222> (107)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (1332)
<223> n equals a,t,g, or c
<400> 408
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<210> 409
<211> 2417
<212> DNA
<213> Homo sapiens
<220>
<221> misc feature
<222> (107)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (680)
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<223> n equals a,t,g, or c
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<400> 409
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<210> 410

<211> 1401

<212> DNA

<213> Homo sapiens

<220>

<221> misc feature

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344

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<222> (1394)
<223> n equals a,t,g, or c
<400> 410
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aaccaacaaa tgtgccagcc aggcaggtat gacagcttac gggactagga ggcatcttta 120
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aaaaaaaaa gggnggccgt t
                                                                1401
<210> 411
<211> 3016
<212> DNA
<213> Homo sapiens
<220>
<221> misc feature
<222> (399)
<223> n equals a,t,g, or c
<400> 411
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ccggggctgg tgattggagg aaaccccgtg tctgcggacg gctgtagcct gtgagcaqcq 120
agatecaggg acagagtete agectegeeg etgetgeege egeegeegee cagagaetge 180
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345

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WO 00/55350

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356

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<210> 425
<211> 1608
<212> DNA
<213> Homo sapiens
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<220>

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<223> n equals a,t,g, or c
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<210> 426
<211> 1794
<212> DNA
<213> Homo sapiens
<220>
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<222> (1789)
<223> n equals a,t,g, or c
<220>
<221> misc feature
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<222> (1790)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (1793)
<223> n equals a,t,g, or c
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<211> 770
<212> DNA
<213> Homo sapiens
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<221> misc feature
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<223> n equals a,t,g, or c
<220>
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<221> misc feature
<222> (40)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (97)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (618)
<223> n equals a,t,g, or c
<400> 427
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taaaaaatag aaattatete actaettaaa teecattttt tteaetteat atgaaagaae 180
atattgatag tatattctat attatttcat agatctgtct gaaagagatt gggaacaaaa 240
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<210> 428
<211> 512
<212> DNA
<213> Homo sapiens
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<222> (30)
<223> n equals a,t,g, or c
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<222> (38)
<223> n equals a,t,g, or c
<220>
<221> misc feature
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<221> misc feature

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<222> (484)
<223> n equals a,t,g, or c
<220>
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<222> (491)
<223> n equals a,t,g, or c
<400> 428
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ccatgaaggg ggtcagtcct acaagattgg tgacacctgg aggagaccac atgagactgg 240
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ggagaagece taccaagget ggatgatggt agattgtact tgcctgggag aargeagegg 420
acgcatcact tgcacttcta gaaatagatg caacgwtcag gacacaagga catctataga 480
attngagaca ncttgagcaa gaaggataat cg
<210> 429
<211> 1470
<212> DNA
<213> Homo sapiens
<220>
<221> misc feature
<222> (1346)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (1347)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (1357)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (1387)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (1415)
<223> n equals a,t,g, or c
<220>
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<222> (1454)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (1462)
<223> n equals a,t,g, or c
<400> 429
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<210> 430
<211> 434
<212> DNA
<213> Homo sapiens
<400> 430
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gaatccccag cccggaaget ctcccagtcc ttcgcccttc ctgttacggg aggeactgtt 180
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<210> 431

<211> 1823

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<212> DNA
<213> Homo sapiens
<220>
<221> misc feature
<222> (1804)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (1805)
<223> n equals a,t,q, or c
<220>
<221> misc feature
<222> (1815)
<223> n equals a,t,g, or c
<400> 431
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<210> 432

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<211> 3391
<212> DNA
<213> Homo sapiens
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<221> misc feature
<222> (1)
<223> n equals a,t,g, or c
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<221> misc feature
<222> (33)
<223> n equals a,t,g, or c
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<222> (68)
<223> n equals a,t,g, or c
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<222> (114)
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<222> (3293)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (3391)
<223> n equals a,t,g, or c
<400> 432
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<222> (476)
<223> n equals a,t,g, or c
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<222> (519)
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<222> (530)
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<223> n equals a,t,g, or c
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<211> 1580
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<213> Homo sapiens
<220>
<221> misc feature
<222> (873)
<223> n equals a,t,g, or c
<400> 440
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<222> (136)
<223> n equals a,t,g, or c
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<222> (462)
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<213> Homo sapiens
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acaagttotg aaataatago acaatttoaa agaagagact otttgcaaag ttgataacat 240
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<211> 1360
<212> DNA
<213> Homo sapiens
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<220>
<221> misc feature
<222> (302)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (330)
<223> n equals a,t,g, or c
<400> 444
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<210> 445
<211> 1835
<212> DNA
<213> Homo sapiens
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<221> misc feature

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<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (1229)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (1738)
<223> n equals a,t,g, or c
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<221> misc feature
<222> (1747)
<223> n equals a,t,g, or c
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<221> misc feature
<222> (1758)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (1801)
<223> n equals a,t,g, or c
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<221> misc feature
<222> (1806)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (1831)
<223> n equals a,t,g, or c
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<212> DNA
<213> Homo sapiens
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<221> misc feature
<222> (55)
<223> n equals a,t,g, or c
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<211> 375
<212> DNA
<213> Homo sapiens
<220>
<221> misc feature
<222> (153)
<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (313)
<223> n equals a,t,g, or c
<400> 447
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<210> 448
<211> 1393
<212> DNA
<213> Homo sapiens
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<221> misc feature
<222> (1360)
<223> n equals a,t,g, or c
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<221> misc feature
<222> (1383)
<223> n equals a,t,g, or c
<400> 448
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aaaaaaaaaa aaaaaagggg cgggccgctc taagagggtn ccctcgaggg gggcccaagn 3180
tttacgcggg gcatgccgac gt
                                                                   3202
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<211> 941
<212> DNA
<213> Homo sapiens
<220>
<221> misc feature
<222> (927)
<223> n equals a,t,g, or c
<400> 472
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atataggttg taatgaaact gtagtctcag ttggaagcct agacatgaaa tgggtcagtg 120
agcaaggete tatteetagt etceageeat geetgtggea acetgageee geteteagea 180
cattggaccc aggcagatgy aaaaaattca cagaactatg atttggactc aagggtttgt 240
agatttcctc cttcattcta atttcagtgt ctaaaattct tgcatccrtg aacgagctgg 300
gcatttgatg agacagggcy gaatactgca gttttcctcc tagaaatcmt ctggggcatt 360
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aagaatatca tgaccagett teaggeetee tgaagtatat eteteacatt gteetgttet 540
catgctgagg agcctgagat ccctgtgtgg ggattagaca gtggactgtt atgggtgtag 600
gtgaattggc ttattttgtc tgtccctgtc tgaatgtatt gcaggaatta aaaaggacca 660
agaagaggaa gaagaccaag gcccaccatg ccccaggctc agcagggagc tgctggaggt 720
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tettgwaaca geetgaetee tgeewgeeet ayrgaagtte ettttatgea ttggaggaaa 840
aacatgttgg cttttctctt ggacgtggga gaaattgaaa agaaggggaa ggggaagaaa 900
agaaggggaa gaagatcaaa gaagganaga agaaggggac g
                                                                  941
<210> 473
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<210> 4/3 <211> 1279

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<213> Homo sapiens
<220>
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<223> n equals a,t,g, or c
<220>
<221> misc feature
<222> (1273)
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aaagaagagt ttctccagag gaagcaatca aatgtgaaga caaattcaca aaatccaaaa 180
ctgtttatag cattcttcgt catgttgctg aggtgttaga atacaccaag gatgagcagc 240
tggaaagcct attccagagg actgcctggg tctttgatga caagtacaag agacctggat 300
atggtgccta tgatgcattt aagcatgcag tctcagaccc atctattttg gatagtttag 360
atttgaatga agatgaacgg gaagtactca ttaataatat taataggcgc ttgaccccac 420
aggotgtcaa aattogagoa gatattgaag tggottgtta tggttatgaa ggcattgatg 480
ctgtaaaaga agccctaaga gcaggtttga attgttctac agaaaacatg cccattaaga 540
ttaatctaat agctcctcct cggtatgtaa tgactacgac aaccctggag agaacagaag 600
gcctttctgt cctcagtcaa gctatggctg ttatcaaaga gaagattgag gaaaagaggg 660
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cacagagcag cgcttcctgg ctgtaaatcc tagacttgaa agttttccag tattgaaaac 900
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gaaccaagtt tantttggg
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<211> 3209
<212> DNA
<213> Homo sapiens
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<221> misc feature
<222> (427)
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gacctggtct tgaactcctt ggcggaagag aagctgcarg ccagcgtgag gtgcttggct 120
acgcacggtc gcttcctgga aattggcaaa ttcgaccttt ctcagaacca mccgctcggc 180
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